SOLUBLE ORGANIC NITROGEN IN PLANT AND SOIL PROCESSES IN THE UPPER COASTAL PLAIN

by

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(Under the Direction of Rebecca R. Sharitz and Christopher S. Romanek)

ABSTRACT

Primary productivity in many terrestrial ecosystems is limited by nitrogen (N) availability, so plant uptake of soluble organic N (SON, free amino-N) compounds is potentially important in plant N nutrition and ecosystem N cycling in Upper Coastal Plain ecosystems. The potential effects of plant organic N uptake on ecosystem processes were initially explored in floodplain ecosystems using a nutrient model that incorporated a plant-available organic N pool. The model predicted that short-term organic N uptake by plants was most affected by competition with soil microorganisms for available N, while longer-term organic N uptake was more strongly affected by soil physicochemical properties. In a two-year field survey of four major ecosystem types in the Upper Coastal Plain (floodplain, upland hardwood, oldfield, sandhills), seasonal concentrations of soluble organic and inorganic N were highly variable in both forest floor and mineral soils of all ecosystem types and were strongly related to litterfall and rainfall events. Concentrations of soluble N were greatest in forest floors and decreased with soil depth, and the release of N from forest floors strongly influenced N concentrations in the

mineral soils of all ecosystems. On a landscape scale, soluble organic and inorganic N concentrations were correlated to ecosystem characteristics reflecting broad environmental gradients. In a field study using stable isotope tracer techniques, roots of both *Acer rubrum* L. and *Pinus palustris* Miller demonstrated the ability to compete well against soil N immobilization factors for available ammonium-N in floodplain and sandhills soils, respectively, while they did not use N or C applied as the amino acid glycine. The relationships between soil N availability and plant N uptake were highly variable and differed over time, reflecting the complexity of plant N use patterns. While organic N uptake as glycine was not unequivocally traced in either tree species examined here, soil soluble organic N clearly plays a pivotal role in soil N dynamics and plant N nutrition in these two contrasting temperate ecosystems.

INDEX WORDS: soluble organic nitrogen, *Acer rubrum*, *Pinus palustris*, stable isotopes

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CHAPTER 1

GENERAL INTRODUCTION[1]

[1] Jin, V. L.

Primary productivity in many terrestrial ecosystems is often limited by nitrogen (N) availability, so plant uptake of soluble organic N (SON, free amino-N) compounds in addition to soluble inorganic N compounds (ammonium, NH_4^+; nitrate, NO_3^-) is potentially important in plant N nutrition and ecosystem N cycling. While *in situ* uptake of amino acids is documented in various plant species growing in alpine and Arctic soils where SON commonly constitutes a larger proportion of total soil N, far fewer studies have examined either plant SON uptake or its relationship to SON availability in warm temperate ecosystem soils. Furthermore, it is unclear how soluble organic and inorganic N availabilities in the soil shift with changing spatial and temporal scales, both individually and in relation to one another. The availability of soil SON for plant use is further influenced by competition with soil microorganisms, and while both intact and excised plant roots readily take up amino acids from hydroponic solutions, the ability of plant roots to compete with soil microorganisms for SON in the field remains unclear.
To address the potential use of SON by plants in warm temperate ecosystems and its effect in ecosystem processes, I selected three sites at each of four contrasting Upper Coastal Plain ecosystems: bottomland hardwood (floodplain) forest, upland hardwood forest, abandoned agricultural field (oldfield), and longleaf pine/scrub oak (sandhills) forest.

The first chapter of this dissertation provides a general overview of the research that has been conducted to date regarding plant organic N uptake in both controlled laboratory settings and in the field. The role of amino acids in plant nutrition, transport mechanisms in the cell membrane, and the role of root mycorrhizas in SON uptake are discussed. In addition, the chapter also describes the sources of soil SON, soil factors influencing competition for N between plant roots and soil microorganisms, and concludes with the potential implications of plant SON uptake in terrestrial ecosystem processes and anthropogenic climate change.

In the second chapter, the potential effects of plant organic N uptake on various large-scale ecosystem processes were explored using a systems model that incorporated a plant-available organic N pool. Of the four ecosystem types studied in this dissertation, floodplain soils were the most organic and, thus, the most likely site at which plant organic N uptake occurred. Because plant organic N uptake potentially occurs in any N-limited ecosystem, evaluating organic N utilization by wetland plants should provide insight into various ecosystem processes, including N dynamics, soil microbial activity, and productivity. Specifically, our objectives were to (1) identify if floodplain forests required organic N to support growth, (2) quantify the effect of organic N uptake on plant and microbial C and N pools, and (3) determine the plant, microbial, and soil factors most affecting plant organic N uptake.

The third chapter provides an assessment of spatial and temporal patterns of soluble organic and inorganic N availability in the litter layer and surface mineral soils of the four ecosystem types listed previously. Both litter layer (entire O horizon) and surface mineral soils (0-5 cm, 5-20 cm) were sampled seasonally for two years at each of the twelve sites to determine the concentrations and relative distributions of soluble organic and inorganic N. In addition to measuring soil soluble N concentrations, litter layer mass, total C, total N, pH, soil moisture content, temperature, and percent clay+silt were measured to assess potential correlations between soil soluble N availability and soil physicochemical characteristics. Temporal patterns in soluble N availability were evaluated at the ecosystem scale through the vertical soil profile for each ecosystem type, and landscape patterns were also assessed to describe the effects of regional factors (temperature, precipitation, and soil physicochemical factors) on overall N availability.

Finally, the last chapter presents data examining plant uptake of SON and inorganic N in the field and the relationships between uptake rates and soil N availability. Soil N availability was characterized by determining both soluble N concentrations (soluble organic N, ammonium-N, nitrate-N) and by calculating the production/consumption rates of these N compounds using stable isotope pool dilution techniques. Plant *in situ* uptake of organic and mineral N was assessed by injecting solutions containing stable isotope-labeled ^{15}N-[2]-^{13}C-glycine and ^{15}N-ammonium sulfate into the rhizospheres of seedlings of each species and comparing the isotope compositions of treated roots and shoots with background tissue values for each species. The relationship between patterns in soil N availability (concentration and supply rate) and plant N uptake characteristics are discussed.

CHAPTER 2

ORGANIC NITROGEN UPTAKE BY PLANTS AND IMPLICATIONS FOR TERRESTRIAL ECOSYSTEMS[1]

[1]Jin, V.L., R. D. Evans, and R.R. Sharitz. To be submitted to *Plant and Soil*.

INTRODUCTION

While much research has addressed the roles of inorganic nitrogen (N) in plant growth and terrestrial ecosystem processes, the contributions of external organic N to overall plant nutrition and ecosystem nutrient cycling have recently received greater attention. Various studies published in the last fifteen years in both agricultural and ecological fields have focused on plant root physiology, primarily uptake mechanisms and symbiotic root associations, and have shown that organic N can act as a significant N source for plant growth. The assumption is still widely held that plant-available N predominantly occurs as the inorganic N (ammonium, nitrate) remaining after the net mineralization of organic matter via microbial decomposition (Schimel & Chapin, 1996; Lipson & Monson, 1998). In some ecosystems, however, net mineralization rates account for less than 50% of the total annual N taken up for biomass production (Nadelhoffer *et al*., 1992; Jonasson *et al*., 1996). While N supply rate, soil buffer capacity, diffusion rate, and root density also influence plant inorganic N availability (Leadley *et al*., 1997), other N sources such as low molecular weight organic N in the form of free amino acids (AAs) may also be used for plant growth (Abuzinadah *et al*., 1986). Soluble forms of organic N such as AAs and proteins can be present in concentrations similar to or greater than those of mineral N in soils (Schobert & Komor, 1987; Kielland, 1994; Turnbull *et al*., 1996; Kielland, 1997), and plant uptake of these organic N forms could thus play important roles in plant nutrition, ecosystem productivity, and terrestrial N cycling in various ecosystems.

As early as 1894 in one of the earliest descriptions of mycorrhizal symbiosis, A. B. Frank (Frank 1894) suggested that organic N could be an important N source for plant nutrition. Traditional studies from agriculture also have long shown that adding organic residues to soils can improve crop yields, but the contribution of organic N to increased productivity has often

been limited to its role in increasing N mineralization by relaxing microbial carbon (C) limitations (Mengel, 1996). More recent publications in agriculture have shown direct relationships between increased yields of some plant species and the preferential uptake of organic N from added organic residues (Yamagata *et al*., 1996; Matsumoto *et al*., 2000).

The number and variety of studies of plant organic N uptake have steadily increased, especially since the mid-1980s. Thus far, studies have focused on the occurrence of species-specific plant organic N uptake, the physiological mechanisms of organic N uptake, the influence of mycorrhizas on plant access to soil organic N, or rhizosphere competition between microbes and plant roots for organic N sources. Although the number of studies on plant organic N use is increasing, the potential contributions of organic N to terrestrial ecosystem productivity and the ecological implications for other ecosystem processes have yet to be elucidated empirically. The objectives of this review are to summarize the published studies on plant organic N uptake (particularly as AAs), and discuss the possible influences of a bioavailable organic N pool on ecosystem processes and the implications of such a pool under anthropogenic changes to the environment.

AMINO ACIDS IN PLANT NUTRITIONS

In general, plants derive N from four major sources: internal recycling, symbiotic fixation of atmospheric N, direct uptake from soil via roots, and indirect uptake from soil via mycorrhizas (Hobbie *et al*., 2000). Studies of the mechanisms of plant acquisition and translocation for each of these sources have heavily concentrated on the uptake of mineral N as the primary bioavailable N pool (or gaseous N, in the case of symbiotic fixation) after which inorganic N is transformed within the plant to organic forms. Amino acids and amides are the first stable

products of inorganic N assimilation and are the predominant forms of N that are metabolically available (Bush, 1993; Barneix & Causin, 1996). In addition to supporting plant growth, concentrations of different AAs in plant tissues help regulate various metabolic processes, including the uptake of exogenous mineral N, enzyme synthesis and activity, rates of symbiotic N fixation, C accumulation, leaf turgor, leaf senescence, and N remobilization (Chapin *et al*., 1990; Barniex & Causin, 1996).

In many plants, high concentrations of AAs and amides can accumulate in various vegetative structures, such as leaf vacuoles (Boudet *et al*., 1981). Storage AAs are typically arginine, glutamine, and asparagine, and can compose a larger proportion of total plant N than of total C (Chapin *et al*., 1990; Nordin & Näsholm, 1997; Fischer *et al*., 1998). In addition, the recycling of AAs between roots and shoots provides a dynamic N resource, the size of which depends on the balance between the plant capacity to absorb and assimilate nitrate and remobilize other N stores (Nordin & N·sholm, 1997).

PLANT ORGANIC N UPTAKE

Amino acids are co-transported into cells through the plasma membrane by both general and AA-specific proton-coupled symports which are pH specific, mulitphasic, and are ubiquitous to most cellular and subcellular membranes (Kinraide & Etherton, 1980; Schobert & Komor, 1987; Bush, 1993; Heilmeier *et al*., 2000). Since AA transporters are common features of cell membranes, transporters found in root cells can provide access to soil organic N sources (Fischer *et al*., 1998). Previous studies of non-protein AA (*i.e.* phytosiderophore) uptake have shown that a variety of high-affinity AA transporters are present along the entire root length (Jones *et al*., 1994; Lipson & Monson, 1998) and can readily take up AA molecules up to 43.8 kDa in size

(von Wiren *et al.*, 1997). Additionally, any low molecular weight compounds that are released into the rhizosphere via passive diffusion across the plasmalemma could also be recaptured by active uptake (Jones & Darrah, 1994; Lipson & Monson, 1998). Although kinetic uptake parameters for both organic and inorganic N solutes are very similar (Jones & Darrah, 1994), little interaction occurs between these uptake systems (Schobert & Komor, 1987; Turnbull *et al*., 1996). Therefore, regardless of mineral N availability or levels of mineral N uptake, plant uptake of AAs is likely to act as a general mechanism for N acquisition (Jones & Darrah, 1994; Turnbull *et al*., 1996; Lipson & Monson, 1998).

Because amino acid uptake co-transporters are strongly pH dependent (Schiller *et al*., 1998; Falkengren-Grerup *et al*., 2000), plants in ecosystems with more acidic soils are mechanistically more likely to take up organic N than plants in more alkaline soils. For glycine, one of the most extensively studied AAs in plant uptake, rates can be 10-times higher at pH 6 than at pH 10 (Schiller *et al*., 1998). In a study of Cyperaceae, 12 of 13 species took up glycine at higher levels in more acidic soils (pH = 5.2) than in circum-neutral soils (pH = 7.5; Raab *et al*., 1999).

Studies have reported that the forms of N preferentially taken up are correlated with the abundances of these N forms in the soil (Waring & Schlesinger, 1980; Nordin *et al.,* 2001). High abundances of AAs have been found in acid organic soils where low temperatures or flood-induced anoxia limit decomposition and N mineralization, and soil organic N concentrations often exceed those of inorganic N (Kielland, 1995; N·sholm & Persson, 2001). For example, net N mineralization in tundra ecosystems frequently supplies only 10% of the annual N uptake by plants and could be negative during the period of plant growth (Nadelhoffer *et al*., 1992; Jonasson *et al*., 1996). Studies of whole plants and excised roots from these and similar arctic,

boreal, and heath-type ecosystems have shown that plant AA uptake rates can equal or exceed rates of mineral N uptake (Table 2.1) and can comprise 10-82% of annual N uptake (Kielland, 1994; Turnbull *et al*., 1996; N·sholm *et al*., 1998; Nordin *et al.*, 2001). Other researchers have found that some plant species exhibit preference for organic N through either higher biomass accumulation or greater total plant N concentrations when grown on organic compared to inorganic N sources (Majerowicz *et al*., 2000; Schiller *et al*., 1998).

Most plant species (probably >90%; Smith & Read, 1997) form mycorrhizal associations, and many studies have demonstrated the active roles of vesicular-arbuscular, ericoid, orchid, and ectomycorrhizas in general plant N acquisition (Stribley & Read, 1980; Smith & Read, 1997; Majerowicz *et al*., 2000). In the case of organic N uptake, mycorrhizal fungi can directly take up simple organic molecules like AAs by producing proteolytic and chitinolytic enzymes that degrade large organic N (Chalot & Brun, 1998). In addition, mycorrhizal association increases the availability of soil nutrients to plants because hyphal mycelia penetrate into soil pores too small for fine root hairs and increase the overall surface area for nutrient absorption (Smith & Read, 1997). The benefits afforded to the plant via its fungal associates will depend on several factors, including plant-fungus compatibility, the competitive ability of the symbiont, and the capacity of the fungus to mobilize organic N to its plant partner (Smith & Read, 1997). After fungal uptake of soil N compounds, the primary route of direct organic N transfer to colonized roots occurs through the extracellular mycelia (Smith & Read, 1997; Jones, 1999). Without their mycorrhizal symbionts, few typically mycorrhizal plant species can continue to access significant quantities of organic N, and these uncolonized plants show significantly slower growth rates than colonized plants (Stribley & Read, 1980; Bajwa & Read, 1986; Schobert & Komor, 1987; Turnbull *et al*., 1996). Despite the strong dependence on mycorrhizas for organic

N uptake, however, many non-mycorrhizal plant species (*i.e.* grasses, species producing proteoid roots) are also capable of taking up significant quantities of soil AAs (Chapin *et al*., 1993; Turnbull *et al*., 1996; Leadley *et al*., 1997; Raab *et al*., 1999).

Recently, stable isotope pulse-chase techniques have been used to demonstrate uptake of organic N substrates. In ^{15}N studies in which the rhizosphere was labeled with ^{15}N glycine, Heilmeier *et al.* (2000) showed a 22x increase in whole plant delta ^{15}N values. Plant uptake rates of ^{15}N-labeled glycine in a wet heathland were typically 30-60% of ammonium uptake, and almost equal for proteoid species (Schmidt & Stewart, 1997). Glycine and serine were extensively metabolized within plants, composing 41% and 37% of the soluble ^{15}N labeled AAs, respectively (Schmidt & Stewart, 1997). Although these studies appear to demonstrate organic N uptake by plant enrichment of ^{15}N, a major difficulty lies in determining if the ^{15}N was assimilated by the plants directly from the labeled AA or from enriched inorganic N mineralized from the label in the rhizosphere. To address this problem, other studies have used doubly-labeled compounds and typically compared enrichment levels of ^{13}C:^{15}N ratio of the plant to the ratio of labeled ^{13}C to ^{15}N in the compound used. In such studies, the uptake of intact glycine molecules was indirectly indicated by equivalent enrichments of ^{13}C and ^{15}N using doubly labeled (*i.e.* ^{15}N, ^{13}C-[2]-glycine; ^{13}C:^{15}N=1) or universally labeled AAs (Lipson & Monson, 1998; Näsholm *et al*., 1998; Lipson *et al*., 1999b; Näsholm *et al*., 2000). However, studies using ^{13}C AAs labeled at the carboxyl C have not demonstrated uptake of both C and N isotopes (Schimel & Chapin, 1996; Hodge *et al.*, 1998, 1999), and authors have noted that these labels could have been lost through respiratory decarboxlyation (Näsholm *et al*., 2000; Tuin & Shelp, 1994).

SOIL AA AVAILABILITY AND MICROBIAL COMPETITION

In contrast to inorganic N, which typically constitutes less than 1% of global terrestrial N, free AAs globally compose 5-50% of the total soil N present and can be the most common forms of organic N in soils (Stevenson, 1982; Senwo & Tabatabai, 1998). Concentrations of these soluble N forms in soils can be similar to or greater than those of mineral N and can vary widely depending on climatic factors, soil chemical and physical characteristics, litter inputs, and soil disturbance events (Schobert & Komor, 1987; Kielland, 1994; Turnbull *et al*., 1996; Sanchez & Lazzari, 1999). Soil microorganisms also use organic N for both N and C demands, so the overall availability of soil AAs to plants could be further influenced by competition with microbes for organic N sources.

Free AAs in soils are derived from microbial cell lysis, the breakdown of organic materials via extracellular enzymes, and the exudation of AAs from plant roots. Reported concentrations of total AAs in soils range from not instrumentally detectable to 1980 $\mu g\ g^{-1}$ dry soil or 158 $\mu mol\ L^{-1}$ soil water (Table 2.2). Even though approximately 140 AAs and AA derivatives have been identified as constituents of living organisms (Stevenson, 1982), in most of these studies, soil AA concentrations were dominated by few specific AAs, but varied depending on ecosystem and soil use histories. For alpine, boreal, and heath-type ecosystems which typically have acid organic soils, the most abundant AAs included serine, glycine, aspartate, glutamate, and arginine (Abuarghub & Read, 1988b; Kielland, 1995; Raab *et al*., 1999; Nordin *et al*., 2001). In subtropical or mixed deciduous forests with less acidic soils, serine, alanine, glycine, aspartate, and leucine were most abundant (Turnbull *et al*., 1996). Glutamine, glutamate, asparagine, aspartate, glycine, and alanine were the dominant AAs in grassland agricultural soils

(Ostle *et al*., 1999; Senwo & Tabatabai, 1998; Beavis & Mott, 1999). Interestingly, a few AAs appear to be common to all of these soils: glycine, alanine, serine, and aspartate.

All of the most commonly found soil AAs appear in the primary routes of plant AA synthesis in which transaminations between glutamate, alanine, and aspartate provide the C and N required in the synthesis of other AAs (Fig. 2.1). In addition, the AAs most often used for xylem and phloem transport are glutamine and asparagine, and to a lesser extent, arginine, glutamate, and aspartate (Lea & Ireland, 1999). Major microbial pathways of AA synthesis also use glutamate, asparatate, serine, and glycine as the primary building blocks for other AAs and proteins. Therefore, it seems likely that the presence of similar AAs in various soils reflects the composition of AAs that are released through plant root exudation, decomposition of plant matter, and microbial cell death. While the presence of similar AAs in many soils might imply that these AAs are not appreciably bioavailable, the importance of these dominant soil AAs in plant and microbial metabolisms suggests that they could be viable organic N sources and that the soil abundances of these AAs simply reflect their metabolic importance in plant tissues and microbial cells and subsequent rapid soil turnover times.

Differences in AA composition and concentrations in various soils could be attributable to other factors that are site-specific, such as soil chemical or physical properties that might differentially influence the immobilization and mobilization of specific AAs in the soil. Although adsorption of AAs to the soil matrix appears to be AA-specific for some soils (Bartlett & Doner, 1988; Jones & Hodge, 1999) but not for others (Jones *et al*., 1994; Raab *et al*., 1999; Kaiser & Zech, 2000), variable rates of adsorption, desorption, and/or soil proteolysis could control the overall availability of soil AAs for biological uptake (Jones *et al*., 1994; Raab *et al*., 1999). While short-term experiments have shown that uptake across the plasmalemma is the rate-

limiting step for biological uptake and not external AA availability (Schiller *et al*., 1998), physical immobilization of organic N may be more significant than microbial immobilization in some ecosystems (Lipson & Monson, 1998). In organic alpine soils, physical immobilization can be so rapid that AAs are converted into complex insoluble organic matter that is not readily available to either microbes or plants (Lipson & Monson, 1998). Even though the retention of AAs in alpine or arctic soils is significantly greater than mineral N (Raab *et al*., 1999), soluble organic N may be an order of magnitude greater than inorganic N (Kielland, 1997). Some studies have also found positive correlations between AA retention and soil organic matter (Raab *et al*., 1999), but AA availability could also depend on pH, microbial activity, litter input quality, and clay content (Schnitzer *et al.*, 1974; Senwo & Tabatabai, 1998).

Seasonality, the frequency and duration of disturbance events, or land management practices could also influence soil AA concentrations and composition. Seasonal patterns of AA concentration and composition in soil water have been found in arctic and alpine soils (Kielland, 1995; Raab *et al*., 1999; Nordin *et al*., 2001), and could reflect seasonal changes in temperature, moisture, and subsequent biological activity. Disturbances such as drying and rewetting events, fires, and flooding may or may not be seasonally dependent, but can lead to changes in soil AA concentrations (Turnbull *et al*., 1996; Schmidt & Stewart, 1997; Lipson & Monson, 1998; Sanchez & Lazzari, 1999). Land management practices, such as fertilization with manure or crop rotations, can also affect total AA concentrations with AA-specific responses (Beavis & Mott, 1996, 1999; Senwo & Tabatabai, 1998). However, arable soils tend to have lower overall total AA concentrations compared to non-agricultural soils (Ostle *et al*., 1999; Beavis & Mott, 1999).

Although AA concentrations in various soils suggest that AAs are bioavailable to both plants and microbes, the supply rate of the organic N pool (*i.e*. the AA turnover time) may serve

as a better indicator of organic N availability than its instantaneous soil concentration. Depending on soil type and environmental conditions, turnover rates for various AAs range from less than 1 hour to 12 hours (Jones *et al*., 1994; Kielland, 1995; Lipson & Monson, 1998; Jones, 1999; Owen & Jones, 2001), and are likely to be much shorter in rhizosphere soils than in bulk soils (Jones, 1999). Amino acid turnover rates are much more rapid than reported turnover rates of mineral N pools (ammonium, ~1 day, Jackson *et al*., 1989; nitrate ~15 h, Stark & Hart, 1997), and competition for AAs between plants and microbes would presumably be much greater. In addition, the diffusion rate of AAs in soils could be as slow as 1 mm in 24 hours or slower, so externally-derived soil AAs will most likely be consumed wherever they are released and are not likely to laterally diffuse through the soil to plant roots (Owen & Jones, 2001).

Ultimately, the availability of soil AAs for plant uptake will depend on the ability of plants to compete for these organic N sources against soil microbes in the rhizosphere. Although studies using axenic plant cultures are useful in examining uptake mechanisms and utilization of organic N, plants in the field experience very different conditions in the rhizosphere where a mixed population of heterotrophs abound and competition for resources is more intense. In a study using wheat (*Triticum aestivum* cv. 'Atlas') grown in sterile soil, plants took up all three of the AAs added (lysine, glycine, glutamate) and incorporated 60% of the added AAs into plant biomass. However, when wheat was grown in non-sterile conditions and roots competed with microorganisms for added AAs, plants took up only 6% of the total added (Owen & Jones, 2001). Similar studies show that other plant species also claim a small proportion (0.9 to 12 %) of added AAs in competition with soil microbes (Schimel & Chapin, 1996; Lipson & Monson, 1998; Lipson *et al*., 1999a). Although microorganisms appear to be better short-term competitors for organic N, plants could be better competitors on longer time scales (Jackson *et al*., 1989;

Lipson & Näsholm, 2001). Microbial cells turn over more rapidly than plant roots, therefore releasing organic N back into the soils, whereas plants are able to retain captured N to a greater extent (Hodge *et al*., 2000).

In addition to direct competition, plants and microbes could partition organic N resources such that microorganisms may differentially utilize specific AAs, leaving other AAs to be taken up by plant roots. For example, glycine was found to be a poorer substrate for microbial growth compared to 10 other AAs except serine in alpine meadow soils where *Kobresia myosuroides*, a non-mycorrhizal alpine sedge, took up glycine more rapidly than other AAs (Lipson *et al*., 1999b). Different responses of plant and microbial uptake to soil AA concentrations could also increase AA availability to plants. For instance, as the concentration of total AAs in soils increases, plant uptake of alanine, glutamine, and glycine generally increases while microbial uptake rates of lysine, glutamate, and glycine are significantly depressed (Falkengren-Grerup *et al*., 2000). Finally, the availability of soil AAs could also be influenced by spatial and temporal partitioning of organic N between plant roots and rhizosphere microorganisms, similar to inorganic N partitioning seen between plants and soil microbes in alpine ecosystems (Lipson & Monson, 1998; Jaeger *et al*., 1999).

IMPLICATIONS FOR TERRESTRIAL ECOSYSTEM PROCESSES AND ANTHROPOGENIC CHANGE

Ecosystem-level research concerning organic N has focused primarily on its role in N mineralization and subsequent availability for microbes with the implicit assumption that remaining mineral N is available for plant uptake (Lipson & Näsholm, 2001). Recent publications examining soil organic N in relation to other ecosystem parameters, however, have

found very few relationships between AA mineralization rates and soil microbial activity. For 18 contrasting soils collected from moors, grasslands, mixed deciduous woodlands, and coastal dunes, AA mineralization rates were not correlated with microbial respiration or yield, and rates did not significantly change when C substrates were added (Jones, 1999). Jones and Hodge (1999) also found that 10-fold increases in C substrate had little effect on microbial AA uptake rates of lysine, glutamate, and glycine. These results suggest that AA uptake by microorganisms is not driven by microbial C demands, and that other environmental or cellular factors may be controlling the turnover of AAs in the soil. While AA half-life appears significantly correlated with soil temperature, the temperature effect is soil dependent (Jones, 1999), and other soil characteristics such as organic matter content, gross mineralization rates, and concentrations of inorganic N have little influence on the mineralization of AAs (Jones, 1999; Jones & Hodge, 1999). Plant AA uptake similarly does not seem to be correlated with soil C, soil N, soil C:N ratio, inorganic or total N concentrations, or N mineralization rate, but does show a negative correlation with soil acidity and nitrification rate (Falkengren-Grerup *et al*., 2000). Therefore, AA uptake mechanisms appear independent of physiological inorganic N uptake mechanisms as well as exogenous concentrations and fluxes of soil inorganic N.

Plant uptake and incorporation of AAs circumvent the N mineralization steps in biogeochemical N cycling required to supply inorganic N for growth (Chapin *et al*., 1993; Kielland, 1994). Almost two-thirds of the external AAs that are taken up can be incorporated into plant biomass (Owen & Jones, 2001), and in addition to the N gained from using AAs directly from the soil, plants may also supplement their C uptake by using these organic N forms. In one study, up to 9% of total plant C in mycorrhizal birch seedlings (*Betula pendula*) was derived directly from added organic N sources over a 55-day growth period (Abuarghub & Read,

1989). On an ecosystem level, given that organic N constitutes a significant proportion of annual N gain in some ecosystems, some fraction of plant productivity could be attributable to C gained from soil AAs. Assuming that the direct uptake of AAs supplies only 10% of annual plant N gain (a conservative estimate based on published studies of plant AA uptake in various ecosystems thus far) and that 40% of the C in those AAs is respired, C from externally-derived AAs alone could constitute 0.2 to 9.0 g C m^{-2} y^{-1} in various ecosystems (Table 2.3). While the quantity of annual C gained from AAs may be insignificant on an ecosystem level, the demand and contribution of AAs to overall C gain could be more important to individual plants, especially in terms of competitive interactions with other individuals. The developmental stage of the plant or successional stage of the ecosystem might also influence the relative importance of organic N uptake, similar to the use of different mineral N forms (Turnbull *et al.*, 1996; Waring & Schlesinger, 1980). Clearly, investigation of the implications for organic N uptake by plants for both community- (e.g. competition) and ecosystem-level (C and N cycling) processes is further warranted.

Because biological controls of ecosystem N retention depends on both microbial immobilization and on the uptake capacity of the vegetation (BassiriRad, 2000), the effect of anthropogenic N deposition on both plant and microbial N uptake could have significant consequences for ecosystem functioning. The deposition of N compounds originating from industrial and agricultural sources ranges from 1 to 500 kg N ha^{-1} y^{-1}, with average values ranging from 20 to 50 kg N ha^{-1} yr^{-1} (Jeffries & Maron, 1997). Increased availability of inorganic N as well as the acidification due to N deposition could influence plant species composition by altering root ability to compete for N (Aber *et al.*, 1998; Näsholm, 1998). Changes in the timing and location of nutrient uptake and in concentrations of different N sources in the soil could

affect the competitive abilities of plant species (Chapin *et al*., 1998). The effects of N deposition on mycorrhizal functioning could also have consequences to both plant species composition and ecosystem functioning, especially since mycorrhizal assimilation and exudation may be the dominant processes involved in the immobilization of added N (Aber *et al*., 1998). Because increased C demands due to utilization of inorganic N sources might be detrimental for fungal growth, the abilities of different ectomycorrhizal fungi to use organic N sources that provide both N and C might be very important in the field (Wallenda & Kottke, 1998). While long-term N deposition could increase the soil availabilities of both inorganic and organic forms, root inorganic N uptake capacity and mycorrhizal infection rates generally decline in response to increasing long-term N deposition (BassiriRad, 2000; van der Eerden, 1998; Wallenda & Kottke, 1998). Therefore, interactions between the uptake and use of inorganic and organic N sources need further investigation to elucidate how increasing N deposition could affect the utilization of organic N sources by individual species and on an ecosystem level (Wallenda & Kottke, 1998). In addition, plant species diversity in many ecosystems is declining in response to anthropogenic N deposition (Chapin *et al*., 1998). Because higher levels of plant species richness could maximize resource acquisition and ecosystem retention of resources (Chapin *et al*., 1998), the loss of species could impact the partitioning of different N sources, including organic N forms, and subsequently affect ecosystem responses to environmental changes.

Other anthropogenic effects on the environment such as climate change might also influence plant organic N uptake and its effects on ecosystem N and C cycling. Greenhouse gas emissions are predicted to raise mean global temperatures approximately 1.0 to 3.5 ◈C in the next 50 to 100 years (Rustad *et al*., 2001), and subsequent increases in soil temperatures may directly affect the biogeochemical processes regulating N and water availability in the soil

(Pastor & Post, 1988; Mellilo *et al*., 1990). Soil warming could also increase plant nutrient acquisition by enhancing root uptake kinetics (BassiriRad, 2000), and plant species-specific responses of N uptake kinetics could be one of the potential mechanisms by which elevated CO_2 might affect the competitive balance among co-occurring species (Berntson *et al*., 1998). Differential effects of elevated CO_2 on root ammonium and nitrate uptake rates suggest that organic N uptake may also be altered (BassiriRad, 2000), but patterns are not apparent. Plant organic N uptake could also be influenced by elevated CO_2 effects on rates of mycorrhizal development and preferences for different N forms by root symbionts (van der Eerden, 1998). Because there are large C costs to the plant imposed by mycorrhizal colonization (~10-20% of photosynthetically-fixed C in well-colonized plants), increases in CO_2 could alter how mycorrhizas promote plant growth and change rates of C cycling (Fitter *et al*., 2000), especially in relation to soil organic N sources.

Most terrestrial ecosystem studies have focused on mineral N as the primary N source for plant growth and as one of the major factors influencing community species composition, structure, and ecosystem productivity and function (Waring & Schlesinger, 1980). Recent research on plant organic N uptake suggests that traditional paradigms of mineral N roles in community and ecosystem processes should be amended to include an organic N perspective. Plant organic N uptake has been documented in almost every ecosystem where it has been investigated and has also been found in a variety of agricultural and managed landscapes. Because of the apparent ubiquity of plant organic N uptake in terrestrial systems, it is critical to explore the roles of organic N in whole-plant, community, and ecosystem processes to further understand and predict how ecosystems function and respond to natural and anthropogenic changes in the environment.

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Table 2.1. Organic N uptake rates for different ecosystems with various plant species (species names not listed) and comparison to mineral N uptake (AA uptake μmol g d.wt^{-1}h^{-1} unless indicated with * as f.wt.; d.wt = root dry weight, f.wt = root fresh weight, nd = no data).

Ecosystem	AA	AA uptake rate --μmol g d.wt^{-1} h^{-1}--	AA uptake relative to ammonium -----%-----	Reference
Agricultural	Alanine	8.5-14	nd	Jones & Darrah, 1994
		0.36-0.38*	nd	Bright *et al.*, 1983
	Arginine	0.33-0.63*	nd	Bright *et al.*, 1983
	Asparagine	0.20-0.22*	nd	Bright *et al.*, 1983
	Glycine	8-18	nd	Jones & Darrah, 1994
	Glutamate	8.5-13	nd	Jones & Darrah, 1994
		0.047-0.051*	nd	Bright *et al.*, 1983
	Glutamine	8.5-13.5	nd	Jones & Darrah, 1994
	Leucine	6-12	nd	Jones & Darrah, 1994
		0.41-0.46*	nd	Bright *et al.*, 1983
	Lysine	0.46-1.28*	nd	Bright *et al.*, 1983
	Methionine	6-10	nd	Jones & Darrah, 1994
	Ornithine	0.32-0.66*	nd	Bright *et al.*, 1983
	Phenylalanine	5-11	nd	Jones & Darrah, 1994
	Serine	12.5-19	nd	Jones & Darrah, 1994
Alpine meadow	Glycine	2-75	7-135	Raab *et al*., 1999
	Glutamine	2.0-15.6	nd	Falkengren-Grerup *et al.,* 2000
	Alanine	1.5-14.4	nd	Falkengren-Grerup *et al.,* 2000
Dry heath	Aspartate	0.09	7	Kielland, 1994
	Glutamate	0.02	1	Kielland,1994
	Glycine	3.56	253	Kielland, 1994
Dry tropical forest	Glycine	6-37	30-80	Raab *et al*., 1999
Shortgrass steppe	Glycine	35-50	390-670	Raab *et al*., 1999
Shrub tundra	Aspartate	0.06-0.08	7-10	Kielland, 1994
	Glutamate	0.06	6-7	Kielland, 1994
	Glycine	0.28-1.49	30-184	Kielland, 1994
Subalpine fen	Glycine	60	103	Raab *et al*., 1999
Subalpine forest	Glycine	5-10	150-200	Raab *et al*., 1999
Subtropical desert	Glycine	0.01-1.0*	nd	Schiller *et al.*, 1998
	Serine	0.11*	nd	Schiller *et al.*, 1998
Subtropical heath	Glycine	0.6-2.6	26-104	Schmidt & Stewart, 1997
Temperate swamp	Glycine	110	78	Raab *et al*., 1999
Tussock tundra	Aspartate	0-0.11	0-20	Kielland, 1994
	Glutamate	0.03-0.09	5-17	Kielland, 1994
	Glycine	0.5-1.25	94-431	Kielland, 1994
Wet meadow	Aspartate	0.04	4	Kielland, 1994
	Glutamate	0.01-0.02	1-3	Kielland, 1994

Table 2.2. Total minimum and maximum soil AA concentrations for various ecosystems (*growing season average; nd=no detection).

Ecosystem	Depth	Minimum	Maximum	Reference
	--cm--	-----μg g^{-1}dry soil-------		
Agricultural, UK	0-20	350	400	Ostle *et al*., 1999
Agricultural, USA	0-15	573	1384	Senwo & Tabatabai, 1998
Boreal forest, Sweden	0-7	0.81	5.14	Nordin *et al*., 2001
Dry heath, USA	0-10	2.19*	-----	Kielland, 1995
Grassland, UK	0-20	1570	1980	Ostle *et al*., 1999
Moor, UK	0-25	nd	520	Abuarghub & Read, 1988a
Semi-arid grassland, Argentina	0-5	373	861	Sanchez & Lazzari, 1999
Shrub tundra, USA	0-10	2.88*	-----	Kielland, 1995
Tussock tundra, USA	0-10	8.29*	-----	Kielland, 1995
Wet meadow, USA	0-10	1.57*	-----	Kielland, 1995
		----μmol L^{-1} soil water----		
Alpine tundra, USA	10	13	158	Raab *et al*., 1999
Shortgrass steppe, USA	10	25	45	Raab *et al*., 1999
Subalpine fen, USA	10	15	20	Raab *et al*., 1999
Subtropical rock pool, Namibia	---	15	40	Schiller *et al*., 1998
Tussock tundra, USA	0-10	105.5*	-----	Chapin *et al.*, 1993
		-----*n*mol g^{-1}dry soil-----		
Eucalyptus forest, Australia	0-5	nd	4.1	Turnbull *et al*., 1996
Subtropical heath, Australia	0-5	2.5	12.7	Turnbull *et al*., 1996

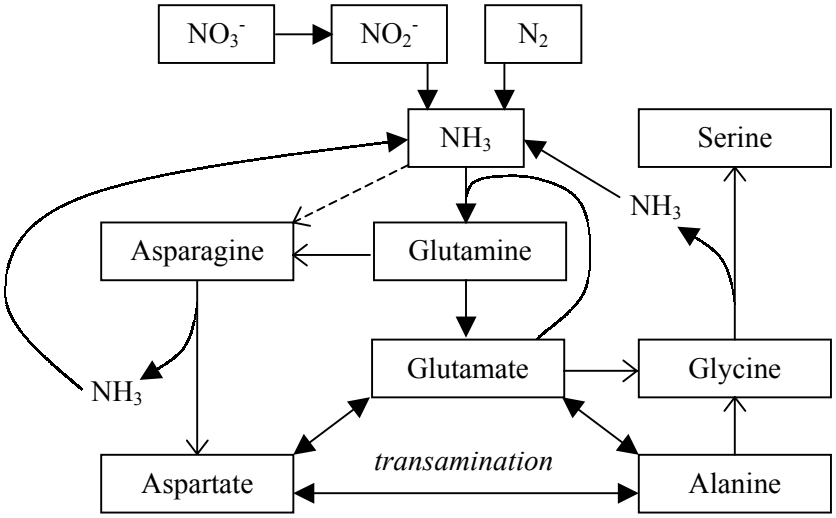
Table 2.3. Potential annual AA-N and AA-C gain in various biomes or ecosystems. Actual numbers given where plant amino acid uptake has been reported. Potential C gain was calculated using the proportion of annual N uptake as AA and assuming: (a) 10% total N uptake from AAs[a] (unless otherwise reported), (b) 40% respiration of AA-C taken up, (c) average C:N ratio =3 for most commonly found soil AAs[b], (d) C:N ratio of new plant biomass of 60[c], and (e) 50% C content of plant biomass[c].

BIOME OR ECOSYSTEM	TNPP[1]	Total N gain	Annual N as AA	Potential AA-N gain	Potential AA-C gain
	-------g m^{-2} y^{-1}-------		----%----	----------g m^{-2} y^{-1}----------	
Cropland	2929	24.4	10	2.4	7.3
Desert	491	4.1	10	0.4	1.2
Forest	1240	10.3	10	1.0	3.1
Grassland	1075	9.0	10	0.9	2.7
Alpine meadow[2,5]		3.3	>50	>1.7	>3.0
Mediterranean	722	6.0	10	0.6	1.8
Pasture	3611	30.1	10	3.0	9.0
Plantation	1757	14.6	10	1.5	4.4
Savanna	1806	15.1	10	1.5	4.5
Tundra	203	1.7	10	0.2	0.5
Dry heath[4]	nd	0.3	72	0.2	0.6
Meadow tundra[4]	nd	0.4	20	0.1	0.2
Moist tundra[3]	nd	0.8–1.6	62	0.5-0.9	1.4-2.8
Shrub tundra[4]	nd	2.3	45	1.0	3.1
Tussock tundra[4]	nd	1.1	60	0.7	2.0
Wetland	973	8.1	10	0.8	2.4

[a]Estimate based on lower range of reported values.
[b]Serine, glycine, asparatate, glutamate, arginine, alanine, leucine, glutamine, asparagine
[c]Waring & Schlesinger, 1980.

[1]Total net primary productivity (g d.wt biomass m^{-2} y^{-1}), Esser *et al.,* 2000
[2]Fisk *et al*., 1998
[3]Chapin *et al*., 1993
[4]Kielland, 1994
[5]Raab *et al*., 1999

Figure 2.1. Primary routes of N in AA synthesis (from Lea & Ireland, 1999).


NO_3^-
NO_2^-
N_2
NH_3
Serine
NH_3
Asparagine
Glutamine
Glutamate
Glycine
NH_3
transamination
Aspartate
Alanine

CHAPTER 3

MODELING ORGANIC NITROGEN UPTAKE BY PLANT AND MICROBIAL COMMUNITIES IN TEMPERATE WETLANDS[1]

[1]Jin, V.L., M.L. Cabrera, and R.R. Sharitz. To be submitted to *Ecosystems*.

ABSTRACT

Plant uptake of soil organic nitrogen (N) has been reported for all ecosystems in which it has been investigated, but most studies have focused on plant-level aspects of organic N use. To investigate the potential ecosystem-level impacts of organic N uptake on both above- and belowground communities, we developed a model for carbon (C) and N cycling in temperate flooded wetlands that incorporates a bioavailable organic N pool. To our knowledge, this is the first ecosystem model that explicitly incorporates plant uptake of organic N. In the model, plant uptake of organic N occurs if mineral N uptake is insufficient to support biomass maintenance and growth, while microbial organic N uptake occurs continuously. Initial input values were derived or extrapolated from field studies of temperate wetlands in the southeastern U.S. Annual simulations show that patterns of organic N uptake varied temporally for both aboveground and belowground communities and that plant organic N uptake occurred in mid- to late summer during peak standing biomass when competition for nutrients is presumably highest. Daily rates of organic N uptake were generally higher for plants (0.00 to 0.59 g m^{-2} d^{-1}) than for microbes (0.08 to 0.21 g m^{-2} d^{-1}), and organic N composed a greater proportion of annual gross N uptake in plants (24.7%) than microbes (1.3%). Sensitivity analyses suggest that plant organic N uptake is more dependent on microbial activity, while microbial organic N uptake is largely independent of changes in plant parameters. Our model supports results from empirical studies suggesting that organic nitrogen could play a significant role in various ecosystem processes.

Keywords: Organic nitrogen uptake, plant-microbial competition, amino acids, temperate wetlands, mean residence times

INTRODUCTION

Nitrogen is one of the most important elements limiting plant growth and ecosystem productivity (Waring and Schlesinger, 1980; Schlesinger, 1997). Typically, mineral nitrogen (NO_3^-, NH_4^+) is considered the main N source for terrestrial plant use (Waring and Schlesinger, 1980; Jaffe, 1992; Schlesinger, 1997), but recent studies have shown that plants from various ecosystems can use organic N forms as well, and sometimes preferentially (Abuzinadah and Read, 1986b; Schobert and Komor, 1987; Kielland, 1994; Schmidt and Stewart, 1997; Raab et al., 1999; Schiller et al., 1998; Heilmeier et al., 2000). Nitrogen budgets calculated for different ecosystems show that the uptake of mineral N alone is inadequate to maintain both standing plant biomass and increases in productivity (Nadelhoffer et al., 1992; Jonasson et al., 1993; Chapin, 1995). Therefore, plants may utilize additional uptake pathways to supply their biological N demand by using various soil organic N forms.

Plant organic N uptake has been shown extensively for species in boreal and alpine ecosystems where decomposition and N mineralization rates are temperature-limited and soil organic N concentrations equal or exceed those of mineral N (Chapin et al., 1993; Kielland, 1994; Schimel and Chapin, 1996; Nasholm et al, 2000). Different plant species in these ecosystems may obtain 10 to 82% of their annual N requirement from soil organic N in the form of amino acids (Chapin et al., 1993; Kielland, 1994). Similar to boreal and alpine ecosystems, temperate wetland soils experience low rates of decomposition and N mineralization due to flood-imposed anoxia (Waring and Schlesinger, 1980; Abuzinadah and Read, 1986b; Jaffe, 1992; Schlesinger, 1997). Since various plant species in temperate wetland ecosystems can also use organic nitrogen (Jin et al., unpublished results), the extent of direct organic N use by wetland

vegetation may have significant implications for whole plant physiology, community dynamics, and ecosystem processes.

In order to explore the potential ecosystem effects of plant organic N uptake on various large-scale processes, we developed a systems model using Stella© that incorporates a plant-available organic N pool, and parameterized the model for temperate wetland ecosystems in the southeastern U.S. Because plant organic N uptake potentially occurs in any N-limited ecosystem, evaluating organic N utilization by wetland plants should provide insight into various ecosystem processes, including N dynamics, soil microbial activity, and productivity. Specifically, our objectives were to (1) identify if plants require organic N to support growth, (2) quantify the effect of organic N uptake on plant and microbial C and N pools, and (3) determine the plant, microbial, and soil factors most affecting plant organic N uptake.

MODEL STRUCTURE

Carbon and Nitrogen Partitioning

The model simulates C and N processes in wetland ecosystems under saturated soil conditions (Fig. 3.1, 3.2). Carbon and N state variables are expressed as g C m^{-2} or g N m^{-2}, and rates are g $m^{-2}d^{-1}$. Boxed compartments represent state variables while arrows represent flows. Flows of C or N are calculated from plant or microbial biomass and C:N ratios except in the case of respiration (CO_2). Initial pool values were estimated as a rough average from field data collected from various wetlands in the southeastern U.S. (Schlesinger, 1977; Duever et al., 1984; Megonigal and Day, 1988; Day and Megonigal, 1993). Other rate constants and initial input values were derived from the literature. State variables, rates, and rate modifiers are summarized in Appendix 3.1.

Plant and microbial carbon is split into two pools: (i) labile C, which is C from easily decomposable material and (ii) humad C, which includes more resistant C, humified C, and adsorbed C (*sensu* McGill et al., 1981). While the entire labile C pool is available for plant and microbial uptake, only 50% of the humad C pool can be utilized for microbial growth (McGill et al., 1981). Carbon dioxide is respired as microbes and plants acquire labile C for growth or through C associated with organic N taken up. The proportions and C:N ratios for plant and microbial inputs to the labile and humad C pools are shown in Table 3.1.

Plant and microbial N is also split into two pools: (i) organic N, which includes N from easily decomposable material and (ii) humad N, which corresponds to more resistant, humified, and adsorbed N. The fraction of the organic N pool that is available for biological uptake ranges from 7 to 23% (Qualls and Haines, 1991; David et al., 1992; Bridgham et al., 1998). Similarly, Laroque and Banton (1996) assume in the Agriflux model that only 10% of the mineral N pool (in this case, the ammonium pool) is available for plant and microbial uptake due to adsorption of ammonium ions to the soil matrix or organic compounds. For our model, we assume that 10% of both the ammonium pool and the organic N pool is available for both plant and microbial uptake. Since nitrate does not appreciably adsorb to the soil matrix in temperate wetland soils and is easily denitrified (Lindau et al., 1994; Ettema et al., 1999), we assumed that uptake of nitrate and denitrification occurred concurrently for the entire nitrate pool.

Ammonium is produced from the mineralization of organic N, and is subsequently nitrified and then denitrified. In our model, microbes use ammonium, nitrate, and organic N pools continuously. Plants prefer ammonium, then nitrate, and use organic N only when mineral N is insufficient to support standing biomass and increases in productivity. Rates of total N uptake by both plants and microbes follow Monod kinetics, and K_m values were converted to a

mass-per-area basis using bulk density (0.45 g cm^{-3}, depth 40 cm; Bowden 1987) and by assuming saturated soil conditions. N uptake rates for both plant and microbes are controlled by their respective C:N ratios. Plant and microbial inputs to the labile and humad N pools parallel C inputs to labile and humad C pools, and are calculated from the rate of carbon transfer and C:N ratios of labile and humad materials of both plant and microbial materials (Table 3.1). Equations for the model are summarized in Appendix 3.2.

Driving variables

The major driving variable for this model is average daily air temperature. We did not use soil moisture content as a driving variable because the model because simulates C and N cycling in wetlands that are inundated six to eight months annually. Thirty-year mean daily temperatures (ºC) (National Climatic Data Center, 1999) were used in one-year simulations, and rate modifiers were used in various model processes to simulate the effect of daily temperature.

The rate of plant net primary production (P, g C m^{-2} d^{-1}) (App. 2) is modified by a temperature factor (T_{PA}), and a seasonal factor (L_D) simulates changes in photosynthetic biomass over the year. T_{PA} ranges from 0 to 1, with the maximum photosynthetic rate occurring at 25 to 35ºC (Larcher, 1995) (Fig. 3.3). L_D describes the annual pattern of foliage present, and ranges from 0 to 1 with maximum foliar biomass present during the months of early-June to the end of July (Fig. 3.4). To simulate nutrient resorption from leaves before senescence occurs, a resorption factor slows the loss of foliar N by resorbing up to 20% of foliar N before leaf fall (Clawson et al., 2001). Rates of plant respiration and uptake of all N forms are also controlled by a temperature factor, T_P, that ranges from 0 to 1 with maximum rates occurring at 25 to 40ºC (McGill et al., 1981) (Fig. 3.6).

Similarly, rates of microbial growth, respiration, and N uptake are controlled by a temperature factor, T_{MGR}, that ranges from 0 to 1 with maximum rates of microbial growth or N uptake occurring between 25 and 40ºC (Fig. 3.6). In contrast to the plant temperature factors that reflect a cessation of activity at 0ºC, microbial activity may continue to occur to levels below freezing (McGill et al., 1981). The temperature minimum for which microbial activity still occurs is set at –5ºC. In addition to the temperature factor, microbial growth is also modified by the microbial efficiency, Y, defining the fraction of available labile C incorporated into microbial biomass.

Because ammonification, nitrification, and denitrification rates are largely microbially-mediated, these rates are also controlled by the temperature factor, T_{MGR}.

MODEL SIMULATIONS

Scenarios

To determine the effect of plant organic N uptake on our modeled ecosystem processes, we ran the model for a baseline one-year simulation that included the organic N pool for plant uptake. We also ran the model excluding the organic N pool for plant use to compare C and N dynamics between the two simulations. In addition, we conducted a sensitivity analysis on the model by readjusting various plant and microbial model components by ± 25% from the baseline simulation. Each component was readjusted individually to determine its effect. Different combinations of modified components were not tested.

Plant organic N uptake

Model simulations using baseline parameters showed that plants did take up organic N during the year when the organic N pool was plant-available. Plants used ammonium and nitrate

concurrently throughout the year, with median daily gross uptake rates of 0.18 g N m^{-2} d^{-1} and 0.09 g N m^{-2} d^{-1}, respectively (Fig. 3.7). Plant organic N uptake occurred for only part of the year, from the beginning of May through mid-September, with a median daily gross uptake rate of 0.26 g N m^{-2} d^{-1} during this time (Fig. 3.7). For the entire one-year simulation, however, the daily median value for plant organic N uptake was zero. When plant organic N uptake did occur, uptake coincided with the active growing season, and peaked when plant standing biomass reached its maximum. Of the total N taken up during the year, ammonium comprised the greatest proportion acquired (47.2%), then nitrate (28.1%), and finally organic N (24.7%). Plants acquired a net amount of 3.6 g N m^{-2} y^{-1} (inclusive of all N forms) and was within the range of total annual plant N uptake reported for various southeastern wetland ecosystems (Peterjohn and Correll, 1984; Walbridge and Lockaby, 1994; Mitsch and Gosselink, 2000). Plant net primary productivity (319 g C m^{-2} y^{-1}) was also within the reported range for this ecosystem type (Megonigal et al., 1997; Mitsch and Gosselink, 2000).

Microbial N uptake also showed that all N forms were used throughout the year, with ammonium, nitrate, and organic N contributing 97.3%, 1.4%, and 1.3% of the total annual N acquired, respectively. Over the one-year simulation, median daily microbial uptake rates of ammonium (12.6 g m^{-2} d^{-1}), nitrate (0.12 g m^{-2} d^{-1}), and organic N (0.16 g m^{-2} d^{-1}) were higher than for plants (Fig. 3.8), and microbes acquired a net amount of 40.9 g N m^{-2} y^{-1}. Microbial C also showed a net gain of 171 g m^{-2} y^{-1}.

Although organic N comprised a much smaller proportion of the total N acquired by microbes than by plants, daily uptake rates were similar for both compartments when plant organic N uptake did occur (Fig. 3.9). When plants and microbes were using organic N resources simultaneously, median rates of uptake by plants were slightly higher than for

microbes (0.26 g m^{-2} and 0.22 g m^{-2}, respectively). Interestingly, peak daily uptake rates for plants occurred somewhat earlier in the growing season (early June) than for microbes (mid-August). This temporal partitioning of peak organic N use in our model suggests that competition between plant and microbial communities for common organic N resources could be lessened during periods of highest biological N demand in either plant or microbial compartments.

Effects of a plant-available organic N pool on other ecosystem parameters

To determine the effects of a plant-available organic N pool on ecosystem parameters, we repeated the baseline simulations and excluded the organic N pool for plant uptake. Furthermore, we compared mean residence times (MRTs) of the different carbon and nitrogen pools for simulations with ($+PN_o$) and without ($-PN_o$) plant organic N uptake (Table 3.2). MRT was calculated as the average of the absolute values of all daily pool sizes divided by the average of the absolute values of all daily pool throughputs. When the organic N pool was not plant-available, both plant pools showed lower annual net gains (244 g C m^{-2} y^{-1} and 2.6 g N m^{-2} y^{-1}) compared to simulations when plant organic N uptake did occur. In addition, plant C and plant N pool MRTs were higher (337 days and 255 days, respectively) in the $-PN_o$ simulation compared to the $+PN_o$ simulation (300 days and 214 days, respectively). This result supports the idea that plant organic N use effectively "short-circuits" the N cycle by circumventing the N mineralization steps necessary for mineral N to become available (Kielland 1993, Chapin et al., 1993).

Interestingly, while microbial C and N MRTs did not differ between the $+PN_o$ and $-PN_o$ simulations, annual net gain of microbial C was lower in the $-PN_o$ simulation (103 g N m^{-2} y^{-1})

although annual net gain of microbial N was not appreciably different (41.0 g N m^{-2} y^{-1}). Because gross microbial C accretion rates were identical for both $+PN_o$ and $-PN_o$ simulations, lower net C in the $-PN_o$ simulation suggests that more C was respired from the microbial community. Higher respiration may have been due to increased competition with plants for organic N resources in the $+PN_o$ simulation.

MRTs from the other ecosystem C and N pools did not differ (labile C, labile N, humad C, humad N, ammonium) (Table 3.2), but nitrate MRT was longer when organic N was not plant-available (11 hours) compared to when organic N was plant-available (<1 hour). Because nitrate uptake kinetics are not changed for either microbial or plant pools between the $+PN_o$ and $-PN_o$ simulations, other ecosystem processes may have influenced the longer MRT for nitrate when organic N was not plant-available. Under this scenario, organic N levels in the soil would be greater than in the $+PN_o$ simulation, allowing more organic N to be ammonified and subsequently nitrified. A greater nitrate influx and unchanged nitrate outflux (through biological uptake) would result in an overall larger nitrate pool size and therefore a longer MRT.

Sensitivity analysis

To assess the influence of other ecosystem parameters on plant organic N uptake, various plant, microbial, and soil parameters were readjusted ±25% from the baseline simulation values (Table 3.3). In general, plant C and N pool values showed stronger responses to changes in plant parameters, whereas microbial C and N pool values were more sensitive to changes in microbial parameters. Although plant C and N values were more sensitive to changes in microbial parameters than vice-versa, microbial C and N values were more sensitive to changes in soil parameters than plant pools.

For plant organic N uptake, the greatest gross annual uptake of organic N occurred when microbial death rate was reduced by 25% (54.7 g m^{-2} y^{-1}), and no plant organic N uptake occurred when the proportion of standing biomass composed of leaves decreased or when microbial death rate increased. Compared to baseline simulation values, plant organic N uptake rate, the number of days during the year when organic N uptake occurred, and the fraction of total gross N comprised of organic N individually appeared to be more sensitive to changes in plant and microbial parameters than soil parameters. For all simulations in which plant organic N uptake occurred, the fraction of total gross annual N taken up as organic N was positively correlated to the number of days that plant organic N uptake occurred ($n=27$, $R^2=0.54$, $p<<0.001$) while the daily uptake rate was negatively correlated ($n=27$, $R^2=0.35$, $p=0.001$). When sensitivity analysis results were examined by changes in plant, microbial, and soil parameters separately, however, the significance of these relationships appeared to be driven primarily by changes in the soil. In addition, for changes in soil parameters only, daily plant organic N uptake rates decreased with increasing number of days when plant organic N uptake occurred ($n=12$, $R^2=0.94$, $p<0.001$). Our results suggest that the rate and temporal extent to which plant uptake of organic N occurs over one year are more sensitive to soil dynamics through soil supply rates of mineral and organic N than to changes in various physiological aspects of either plant or microbial communities.

On a shorter time scale, however, when plant organic N uptake is compared to plant mineral N uptake, changes in microbial parameters have the most significant impacts on the relationships between daily rates of N uptake as well as the proportions of each N form comprising gross annual N gain. Simulation results from manipulating microbial parameters show that as the mean daily plant uptake of organic N increases, plant ammonium and nitrate

uptake both decrease (n=8, R^2=0.72 and 0.67, respectively; p=0.01 for both). The gross annual quantities of ammonium and nitrate acquired subsequently decrease as the quantity of organic N taken up increases. However, the longer-term relationships between the proportions of each N form acquired over the year are significantly affected by changes in both plant and microbial parameters. The negative correlations between proportions of organic N to both ammonium and nitrate have higher r-square values for plant changes (n=10; R^2=0.90, p<0.001; R^2=0.75, p=0.001, respectively) than for microbial changes (n=8; R^2=0.86, p<0.001; R^2=0.65, p=0.01, respectively). These results suggest that mean daily rates and gross quantities of mineral N relative to organic N are more sensitive to changes in microbial parameters, perhaps due to short-term competition between plant roots and microbes. In contrast, changes in both microbial and, more so, plant parameters affect the proportions of mineral N relative to organic N acquired over the longer-term (one year). The absence of soil effects on the relationships between the different N forms taken up by plants further implies that the immediate bioavailability and daily use of each N form is more sensitive to shorter-term biological factors than to soil factors alone.

CONCLUSIONS

Based on our model, we found that organic N uptake in both plant and microbial communities played a substantial role in various ecosystem processes, including nitrogen and carbon cycling times and overall ecosystem productivity. For plants, short-term organic N uptake appeared to be most affected by biological parameters determining N availability, while longer-term organic N uptake was more strongly affected by soil properties. Microbial organic N uptake was largely independent of changes in plant parameters. In addition, sensitivity analyses suggested that C and N dynamics for microbes were relatively less affected by changes in plant

parameters while changes in microbial parameters had a more pronounced effect on plant C and N pools. Overall, our model suggests that for southeastern temperate wetland ecosystems, soil organic N could provide significant amounts of N for both microbial and plant nutrition, and that the role of bioavailable organic N in ecosystem dynamics warrants further study.

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Appendix 3.1. Literature-derived parameter values for state variables, rate constants, rate modifiers, and other values. Estimated parameter values calculated from Jin (unpublished data) and literature values. Assumed values also indicated.

Parameter	Symbol	Units	Initial Value	Source*
STATE VARIABLES				
Plant carbon	P_C	g C m^{-2}	14400	5, 6, 12, 14
Labile soil carbon	C_L	g C m^{-2}	460	Estimated
Humad soil carbon	C_h	g C m^{-2}	22,540	Estimated
Microbial carbon	C_M	g C m^{-2}	400	Estimated
CO_2 evolved	CO_2	g C m^{-2}	0	Assumed
Plant nitrogen	P_N	g N m^{-2}	96	5, 6, 12, 14
Organic soil nitrogen	N_o	g N m^{-2}	15	Estimated
Humad soil nitrogen	N_h	g N m^{-2}	1300	Estimated
Microbial nitrogen	N_M	g N m^{-2}	40	Estimated
Ammonium	N_A	g N m^{-2}	1	8
Nitrate	N_N	g N m^{-2}	0.35	8
RATE CONSTANTS				
Max gross photosynthesis	A_{max}	g C g^{-1}C m^{-2}	0.01	11
Hours of photosynthesis	P_T	h	10	11
Plant root maximum NH_4^+ uptake capacity	V_{maxPA}	g N g^{-1} C d^{-1}	0.0132	11
Plant root NH_4^+ half-saturation constant	K_{mPA}	g N m^{-2}	21.17	11
Plant root maximum NO_3^- uptake capacity	V_{maxPN}	g N g^{-1} C d^{-1}	0.0044	11
Plant root NO_3^- half-saturation constant	K_{mPN}	g N m^{-2}	0.42	11
Microbial maximum C_L uptake capacity	V_{maxL}	g N g^{-1} C d^{-1}	0.07	3

Microbial C_L half-saturation constant	K_{mL}	g N m^{-2}	29	3
Microbial efficiency	Y	-----	0.4	Assumed
Microbial growth constant for C_H uptake	K_{Ch}	d^{-1}	0.01	3
Max microbial death rate	k_{DC}	d^{-1}	0.15	Assumed
Microbial maximum NH_4^+ uptake capacity	V_{maxAM}	g N g^{-1} C d^{-1}	0.075	11
Microbial NH_4^+ half-saturation constant	K_{mAM}	g N m^{-2}	0.2	11
Microbial maximum NO_3^- uptake capacity	V_{maxNM}	g N g^{-1} C d^{-1}	0.075	15
Microbial NO_3^- half-saturation constant	K_{mNM}	g N m^{-2}	1	15
C_L adsorption constant	K_{AL}	d^{-1}	0.003	7
C_h desorption constant	K_{Dh}	d^{-1}	0.00004	7
RATE MODIFIERS				
Temperature factor for plant net primary production	T_{PA}	-----	0 – 1	9, 11
Seasonal factor for foliar biomass present	L_D	-----	0 – 1	8
Temperature factor for microbial growth, respiration, and N uptake	T_{MGR}	-----	0 – 1	9, 11
OTHER VALUES				
Maximum foliar fraction of plant biomass	P_{CL}	-----	0.035	5, 12
Fraction of gross plant C gain respired	f	-----	0.5	9, 16
Root fraction of plant biomass	P_{CR}	-----	0.2	5
Bioavailable fraction of C_L pool	L_{CL}	-----	1	11
Bioavailable fraction of C_h pool	L_{Ch}	-----	0.5	11
Bioavailable fraction of N_o pool	L_{No}	-----	0.1	2, 4, 13
Bioavailable fraction of N_A pool	L_{NA}	-----	0.1	10
Bioavailable fraction of N_N pool	L_{NM}	-----	1	Assumed
Plant biomass C:N ratio	P_{CN}	-----	150	5, 6

C:N ratio of microbial biomass	CN_M	-----	10	11
C:N ratio of labile pool	CN_{CL}	-----	38	Estimated
C:N ratio of humad pool	CN_{Ch}	-----	17	Estimated
Soil bulk density to 40 cm depth	BD	g cm^{-3}	0.45	1
Soil total %C	-----	-----	13	8
Soil total %N	-----	-----	0.75	8
Labile fraction of total soil C	-----	-----	0.02	Estimated
Humad fraction of total soil C	-----	-----	0.98	Estimated
Labile organic fraction of total soil N	-----	-----	0.01	Estimated
Humad organic fraction of total soil N	-----	-----	0.96	Estimated

*1. Bowden, 1987; 2. Bridgham, 1998; 3. Cabrera 1998; 4. David et al., 1992; 5. Day and Megonigal, 1994; 6. Duever et al., 1984; 7. Hassink and Whitmore, 1997; 8. Jin, 2001 *unpublished results*; 9. Larcher, 1995; 10. Laroque and Banton, 1996; 11. McGill et al., 1981; 12. Megonigal and Day, 1988; 13. Qualls and Haines, 1991; 14. Schlesinger, 1978; 15. Vinolas et al. 2001; 16. Waring and Schlesinger, 1980.

Appendix 3.2. Model equations used for various plant, microbial, and soil processes.

Process	Equation
PLANTS	
Net primary production (P, g C m^{-2} d^{-1})	$A_{max} * P_T * (P_C * P_{CL}) * T_{PA} * L_D$
Respiration (R, g C m^{-2} d^{-1})	$P * f * T_P$
Ammonium uptake (U_{Ap}, g N m^{-2} d^{-1})	$(P_C * P_{CR}) * [V_{maxAp} * N_A/(K_{mAp}+N_A)] * T_P$
Nitrate uptake (U_{Np}, g N m^{-2} d^{-1})	$(P_C * P_{CR}) * [V_{maxNp} * N_N/(K_{mNp}+N_N)] * T_P$
Organic N uptake (U_{Op}, g N m^{-2} d^{-1})	$[(P / P_{CN}) - (U_{Ap} + U_{Np})] * T_P$
MICROBES AND SOIL	
Microbial growth	(dC_m/dt, g C m^{-2} d^{-1}) $dC_L/dt + dC_h/dt$
a. from Labile C (dC_L/dt)	$C_M * [V_{maxL} * C_L / (K_{mL}+C_L)] * Y * T_{MGR}$
b. from Humad C (dC_h/dt)	$K_{Ch} * (L_{CL} * C_h) * Y * T_{MGR}$
Respiration (M_R, g C m^{-2} d^{-1})	
a. from Labile C (dR_L/dt)	$(dC_L/dt) / Y * (1-Y)$
b. from Humad C (dR_h/dt)	$(dC_h/dt) / Y * (1-Y)$
Microbial death (M_D, g C m^{-2} d^{-1})	$k_{DC} * C_M$
Ammonium uptake (U_{AM}, g N m^{-2} d^{-1})	$C_M * (V_{maxAM} * N_A / (K_{mAM} + N_A)) * T_{MGR}$
Nitrate uptake (U_{NM}, g N m^{-2} d^{-1})	$C_M * (V_{maxNM} * N_N / (K_{mNM} + N_N)) * T_{MGR}$
Organic N uptake (U_{oM}, g N m^{-2} d^{-1})	$(dC_L/dt) / L_{CN} * T_{MGR}$
Ammonification (dNH4, g N m^{-2} d^{-1})	$(dC_L/dt + dR_L/dt) / L_{CN} * T_{MGR}$
Nitrification (dN03, g N m^{-2} d^{-1})	$N_{max} * T_{MGR}$
Denitrification (dDN, g N m^{-2} d^{-1})	$D_{max} * T_{MGR}$
Soil C adsorption (dA_L/dt, g C m^{-2} d^{-1})	$K_{AL} * C_L$
Soil C desorption (dD_h/dt, g C m^{-2} d^{-1})	$K_{Dh} * C_h$

Table 3.1. Values defining the proportions and C:N ratios of plant and microbial biomass transferred to labile and humad carbon pools.

Substrate	Fraction	Proportion	%C	C:N
Plant litter	Labile	0.05	45	5
	Humad	0.95	50	150
Dead microbial biomass	Labile	0.20	45	3
	Humad	0.80	50	30

Table 3.2. Mean residence times (days) for various carbon and nitrogen pools for model simulations with ($+PN_o$) and without ($-PN_o$) plant-available organic N.

	Carbon		**Nitrogen**	
	$+PN_o$	**$-PN_o$**	**$+PN_o$**	**$-PN_o$**
Plant	300	337	214	255
Microbial	24	24	17	17
Labile	385	385	15	15
Humad	890	890	526	526
Ammonium	----	----	11	11
Nitrate	----	----	0.03	0.45

Table 3.3. Various ecosystem parameters were readjusted ±25% of baseline values to examine the effects on plant and microbial dynamics. Percent differences from baseline values are shown for net annual C and N accretion rates. The number of days that plant organic N uptake occurred and the median uptake value during that time (percent of total gross annual N uptake as organic N) is also shown.

		----------Plants----------				**-------Microbes-------**	
Adjusted parameters		C	N	Org N uptake		C	N
		---% change---		Days	g m^{-2} d^{-1}	----%change----	
PLANT							
Carbon	+	+20%	+20%	146	0.35 (26%)	0	-2%
	-	-25%	-25%	89	0.27 (18%)	0	+2%
C:N	+	+14%	-10%	76	0.40 (15%)	0	0
	-	-19%	+10%	148	0.36 (32%)	0	0
Amax	+	+5%	+6%	144	0.29 (29%)	0	0
	-	-9%	-11%	66	0.40 (15%)	0	0
%Leaf	+	-3%	+5%	144	0.29 (13%)	0	0
	-	-23%	-27%	0	0 (0)	0	0
%Root	+	+9%	+7%	77	0.50 (16%)	0	-2%
	-	-16%	-15%	120	0.34 (27%)	0	+2%
MICROBIAL							
Carbon	+	-11%	-10%	128	0.37 (29%)	-438%	-52%
	-	+13%	+10%	75	0.45 (14%)	+419%	+48%

C:N	+	0	0	0	0 (0)	0	0
	-	0	0	128	0.31 (24%)	+7%	+1%
Y	+	+4%	+2%	152	0.21 (28%)	+490%	+43%
	-	-6%	-5%	115	0.29 (21%)	-410%	-60%
kDC	+	-6%	-9%	0	0 (0)	-473%	-77%
	-	-1%	+1%	156	0.42 (42%)	+709%	+97%
SOIL							
Labile C	+	-1%	-1%	139	0.23 (25%)	+2%	-2%
	-	0	0	124	0.32 (24%)	-4%	+3%
Humad C	+	+11%	+9%	103	0.47 (23%)	+444%	+81%
	-	-9%	-8%	128	0.28 (24%)	-400%	-84%
NH_4^+	+	0	0	134	0.26 (25%)	0	+2%
	-	0	0	134	0.26 (25%)	0	-2%
NO_3^-	+	0	0	134	0.26 (25%)	0	0
	-	0	0	134	0.26 (25%)	0	0
Nitrification	+	+6%	+6%	138	0.28 (24%)	0	-1%
	-	-6%	-6%	130	0.28 (26%)	0	+1%
Denitrification	+	0	0	134	0.26 (25%)	0	0
	-	0	0	134	0.26 (25%)	0	0

Figure 3.1. Compartment diagram for C.

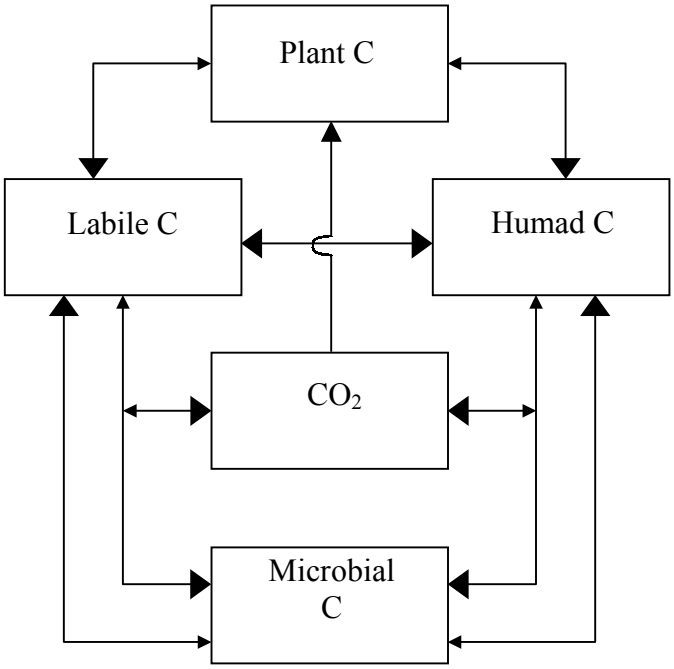

Plant C
Labile C
Humad C
CO_2
Microbial C

Figure 3.2. Compartment diagram for N.

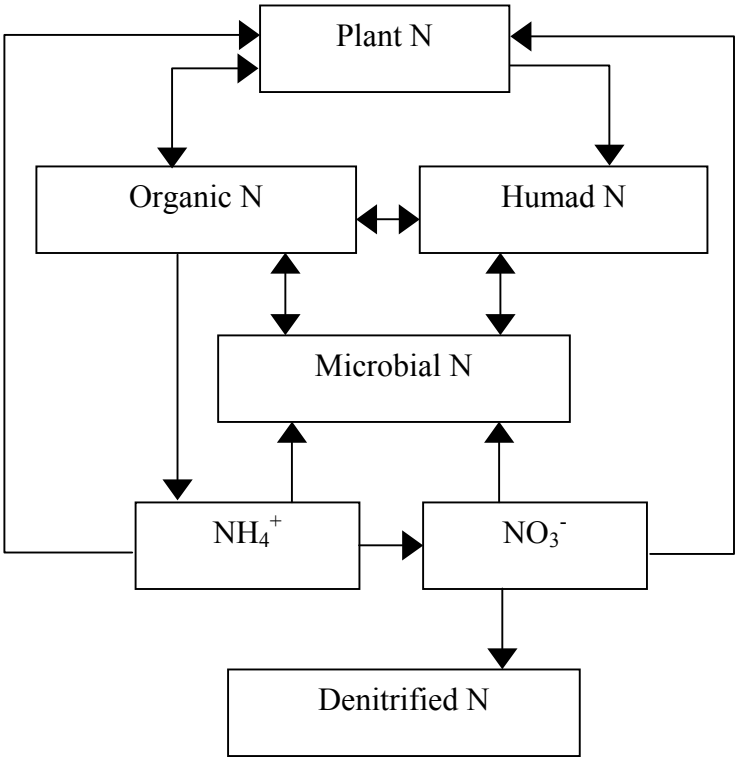

Plant N
Organic N
Humad N
Microbial N
NH_4^+
NO_3^-
Denitrified N

Figure 3.3. Temperature factor for plant net primary production, T_{PA}.

Figure 3.4. Seasonal factor controlling the proportion of photosynthesizing biomass, L_D.

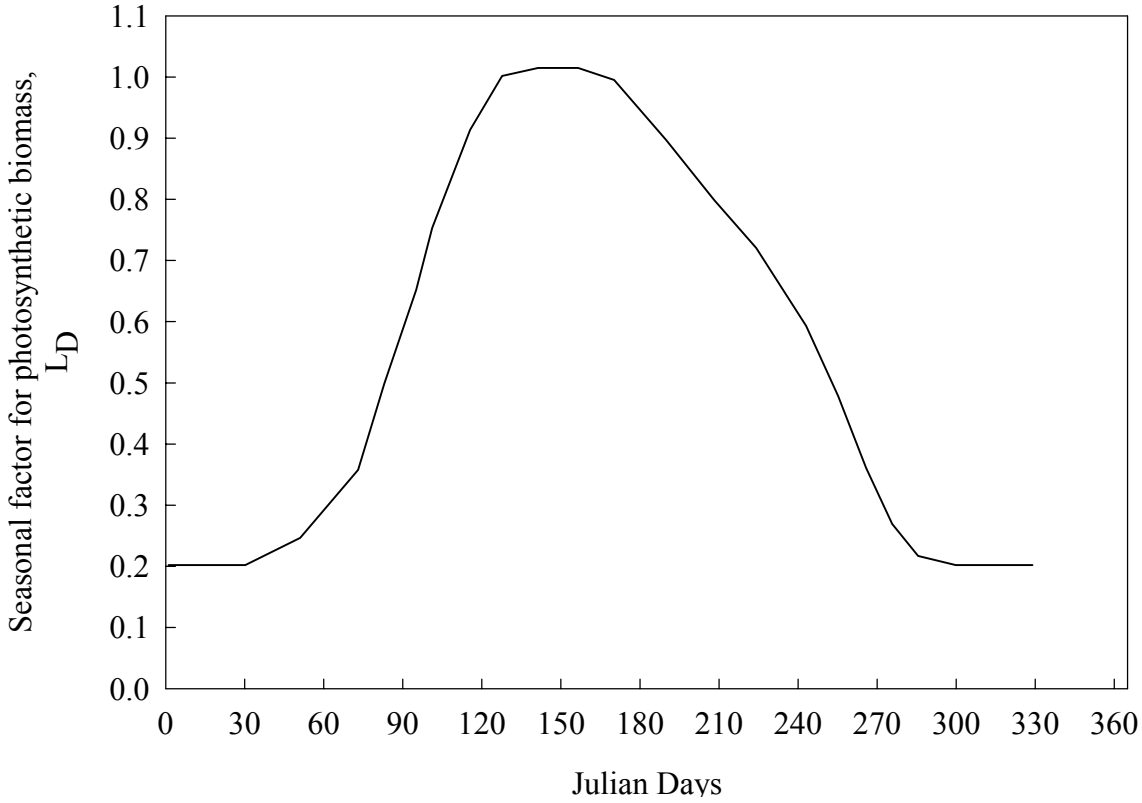

Seasonal factor for photosynthetic biomass, L_D
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
30
60
90
120
150
180
210
240
270
300
330
360
Julian Days

Figure 3.5. Temperature factor for plant respiration and plant N uptake, T_P.

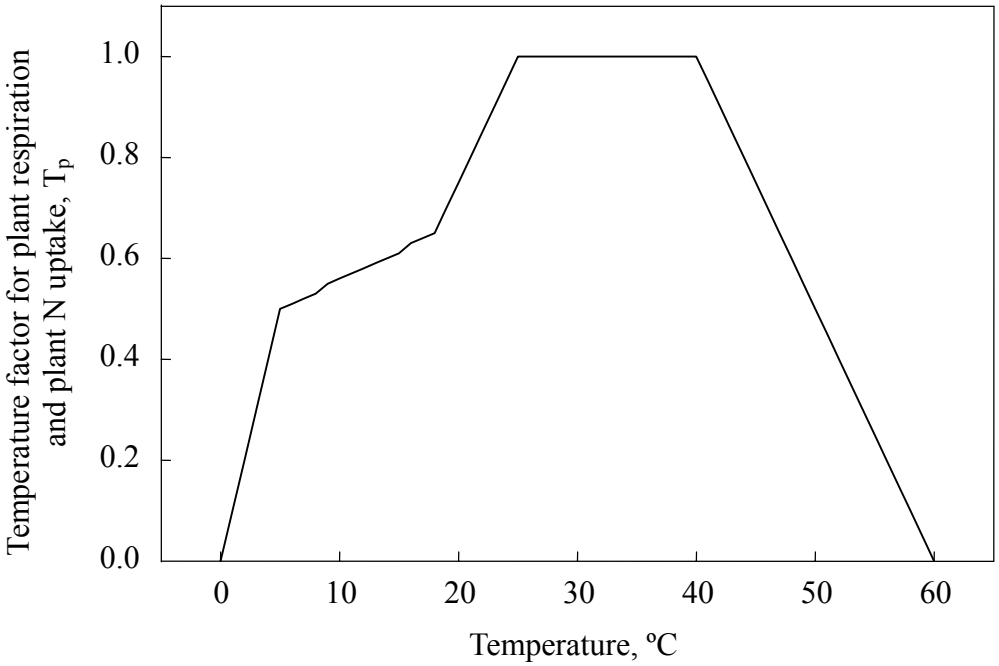

Temperature factor for plant respiration and plant N uptake, T_p
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Temperature, ºC

Figure 3.6. Temperature factor for microbial growth and microbial N uptake, T_{MGR}.

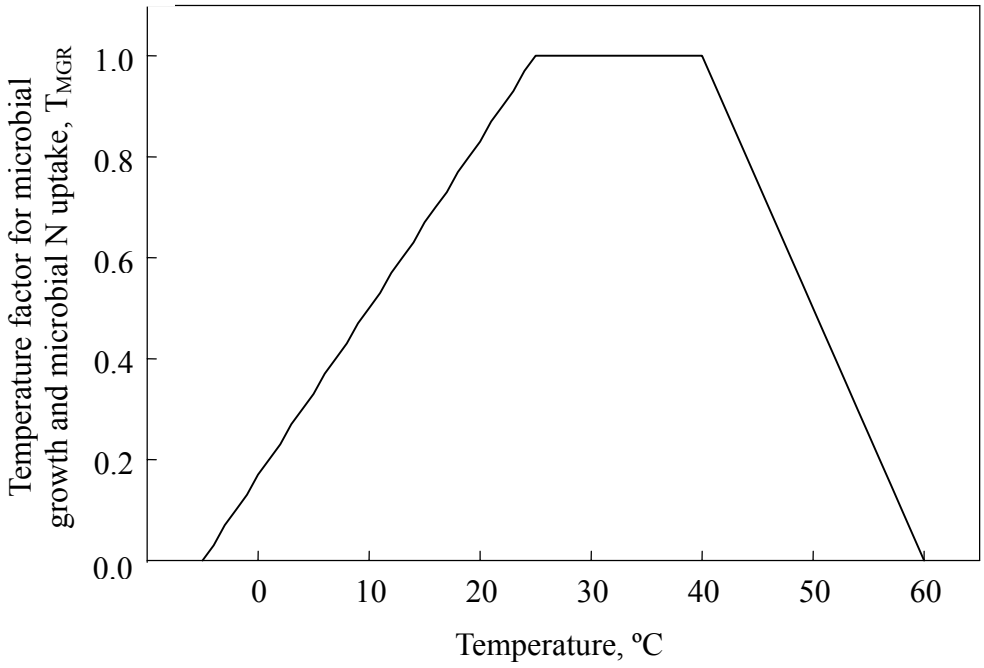

Temperature factor for microbial growth and microbial N uptake, T_{MGR}
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Temperature, ºC

Figure 3.7. Relative frequency distributions for plant gross daily uptake rates of ammonium, nitrate, and organic N. Distributions calculated only for days during which each N form was being used. Median and mean indicated with a solid and dashed line, respectively.

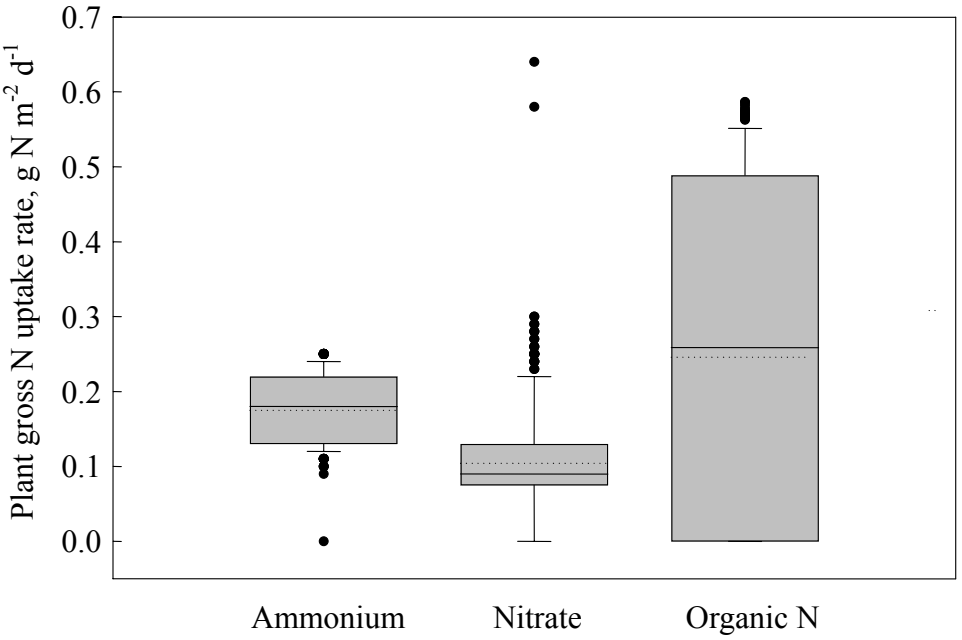

Plant gross N uptake rate, g N m-2 d-1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Ammonium
Nitrate
Organic N

Figure 3.8. Relative frequency distributions for microbial gross daily uptake rates of ammonium, nitrate, and organic N. Distributions calculated for all days of simulation (*i.e.* all forms used each day). Median and mean indicated with a solid and dashed line, respectively.

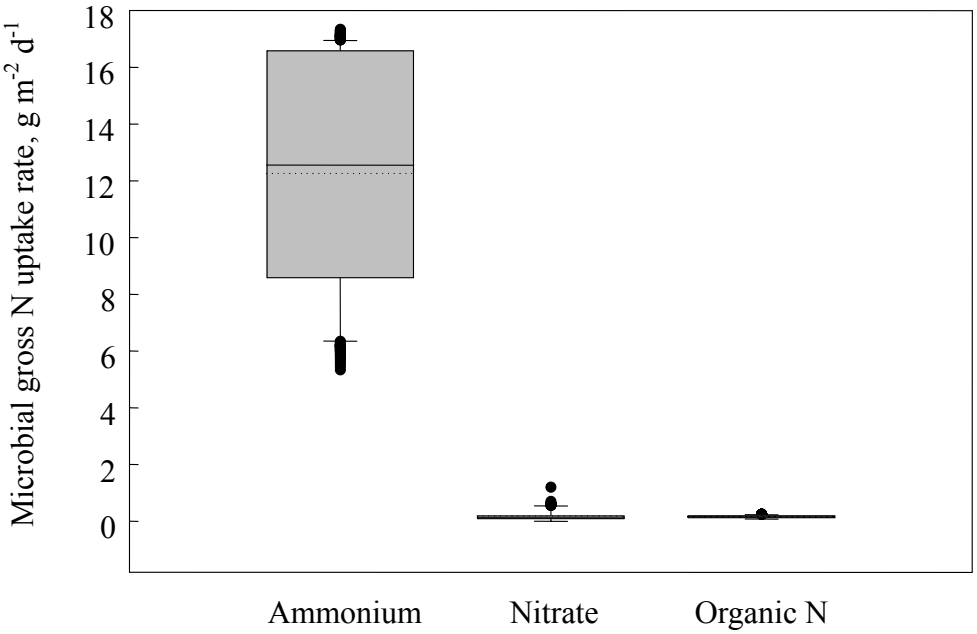
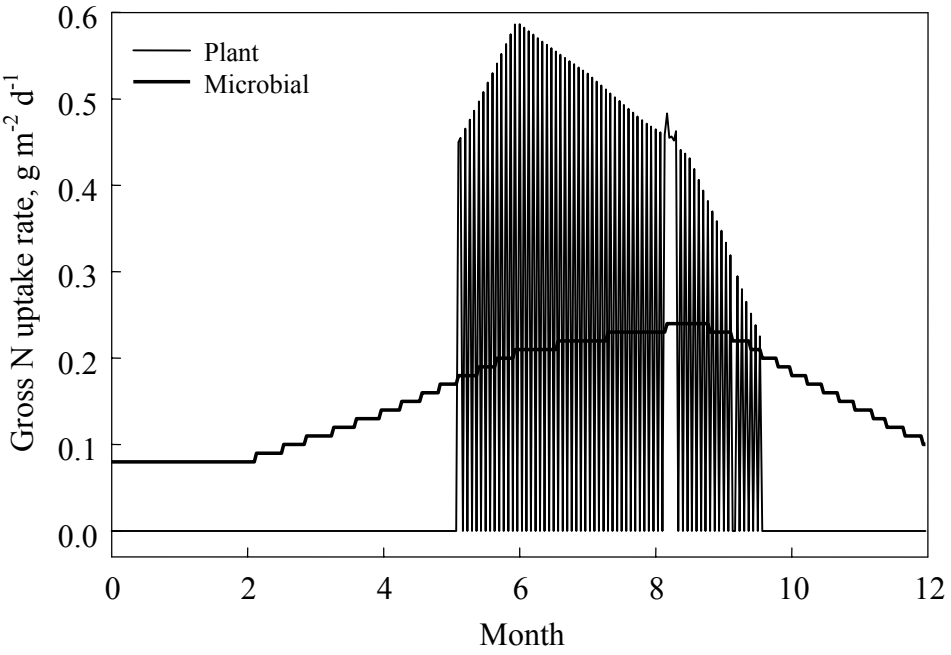

Microbial gross N uptake rate, g m-2 d-1
18
16
14
12
10
8
6
4
2
0
Ammonium
Nitrate
Organic N

Figure 3.9. Gross daily organic N uptake rates for plant and microbial compartments for one-year simulation (Month 0=January).


Plant
Microbial
Gross N uptake rate, g m-2 d-1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
2
4
6
8
10
12
Month

CHAPTER 4

SPATIAL AND TEMPORAL PATTERNS OF SOIL SOLUBLE N AVAILABILITY IN FOUR CONTRASTING UPPER COASTAL PLAIN ECOSYSTEMS[1]

[1]Jin, V.L., R.R. Sharitz and C.S. Romanek. To be submitted to *Soil Science Society of America Journal*.

ABSTRACT

Concentrations of soil soluble organic N (SON; free amino-N) and inorganic N (ammonium-N, nitrate-N) were measured seasonally for two years in twelve soils across four major ecosystem types in the Upper Coastal Plain region of the southeast U.S. The four ecosystems (n=3 sites each) studied were bottomland hardwood (floodplain) forest, upland hardwood (hardwood) forest, abandoned agricultural fields (oldfield), and longleaf pine/scrub oak (sandhills) forest. At the ecosystem level, concentrations and relative availabilities of soluble N compounds were highly variable between sites at each sampling event. Seasonal average values fluctuated temporally, but differences in seasonal total soluble N concentrations were not significant between sampling events at any depth except in the hardwood litter layer and 5-20 cm soils, and the oldfield litter layer. For all ecosystems, average seasonal total soluble N concentrations decreased with depth from litter layer>0-5 cm mineral soil>5-20 cm mineral soil. Soil ammonium-N concentrations were detectable at all seasons, but the presence of SON and nitrate-N varied with respect to ecosystem type, soil depth, and sampling date. Low overall concentrations of SON and nitrate-N during the second year appeared to be correlated with above average monthly rainfall. Very high SON concentrations measured in a few sampling events strongly influenced average seasonal concentrations at all depths in floodplain and hardwood ecosystems and, to a lesser degree, in oldfield and sandhills ecosystems. On a landscape scale using two-year average values from all ecosystems and all depths, both soluble organic and inorganic N concentrations showed significant correlations to other measured soil variables (moisture, soil pH, %clay+silt, soil temperature, total N, total C) that were not apparent at the ecosystem level. Patterns in the overall availability of soluble N compounds, therefore, appeared to correspond with ecosystem characteristics that were strongly influenced by broad

environmental gradients. In addition, the dynamic nature and episodically high concentrations of SON suggest that the soil soluble organic N pool is an important component of the nitrogen cycles in these contrasting Upper Coastal Plain ecosystems.

KEYWORDS: soluble organic N, Upper Coastal Plain, ammonium, nitrate

INTRODUCTION

Almost 95% of total soil nitrogen (N) is associated with soil organic matter and is comprised of a range of simple to complex organic forms (Schulten and Schnitzer 1998). While the more complex of these N compounds are recalcitrant and biologically unavailable, a small pool of soluble organic N is easily mineralizable and so may have a major impact on N cycling dynamics (Appel and Mengel 1993, Murphy *et al*. 2000). Soluble organic N (SON; organic N that is extractable with water, KCl, or other extractant) is composed of nonhumic N-containing substances, such as amino-N compounds (amino acids, amino sugars) (Mengel 1996). In addition to providing N for soil microbial growth (Hart *et al*. 1994, Barraclough 1997), SON is also a major plant-available N source in various ecosystems ranging from arctic tundra to subtropical heathland (Kielland 1994, Atkin 1996, Schmidt and Stewart 1997). In these ecosystems, soil concentrations of SON often equal or exceed those of soluble inorganic N and are positively correlated with rates of plant SON uptake (Nordin *et al.* 2001, Persson and Nasholm 2002, Henry and Jeffries 2003).

Because the SON pool potentially plays a key role in microbial soil N transformations and is correlated with plant organic N uptake, soil SON availability provides some insight into both soil N cycling and plant nutrition. Although the most common index of availability is soil concentration, the relative distribution of SON and inorganic N may also influence N cycling

dynamics. Concentrations and distributions of soluble organic N in soils have been well characterized in cooler ecosystems (Kielland 1995, Atkin 1996) and for agricultural soils (Schulten and Schnitzer 1998, Senwo and Tabatabai 1998, Murphy *et al.* 2000), but little is known about SON concentrations in unmanaged warmer temperate soils, particularly in relation to inorganic N pools. Furthermore, it is unclear how patterns in soluble organic and inorganic N availabilities shift with changing spatial and temporal scales, both individually and in relation to one another.

To evaluate concentrations and distributions of soluble organic and inorganic N compounds in temperate soils, twelve soils from four contrasting ecosystem types in the Upper Coastal Plain region of the southeast U.S. were sampled. Both litter layer (entire O horizon) and surface mineral soils (0-5 cm, 5-20 cm) were sampled seasonally for two years at three sites each in bottomland hardwood forest (floodplain), upland hardwood forest, abandoned agricultural field (oldfield), and longleaf pine/scrub oak forest (sandhills) ecosystems. In addition to measuring soil soluble N concentrations, litter layer mass, total C, total N, pH, soil moisture content, temperature, and percent clay+silt were measured to assess potential correlations between soil soluble N availability and soil physicochemical characteristics.

MATERIALS AND METHODS

Study sites

Soils were sampled from July 2001 through April 2003 at eight sampling events that corresponded to each season. Summer samples were taken when aboveground biomass was approximately greatest. Fall sampling occurred just after the majority of trees had senesced.

Winter samples were taken when aboveground activity was minimal, and spring samples were collected soon after leaf emergence.

At each sampling event, three sites each of bottomland hardwood forest (floodplain), upland hardwood forest, abandoned agricultural field (oldfield), and long-leaf pine/scrub oak (sandhills) forest located on the U.S. Department of Energy's Savannah River Site (SRS; Aiken, SC, USA; Fig. 4.1). The SRS is located on the Upper Atlantic Coastal Plain of south-central South Carolina, and the four ecosystems studied are commonly found in this physiographic province (Workman and McLeod 1990). The climate is humid and temperate with short, mild winters and long, warm summers. Mean daily air temperatures range from 7°C in January to 27°C in July with a mean annual air temperature of 18.2°C (Hunter 1990). The average annual precipitation is approximately 120 cm, and monthly precipitation values corresponding with soil sampling events in this study are shown in Figure 4.2. The following descriptions of the soil and vegetaion of the four ecosystem types are taken from Rogers (1990), Workman and McLeod (1990), and Davis and Janecek (1997).

Soils at floodplain sites are sandy, siliceous, thermic Cumulic Humaquepts (Pickney series) and Fluvaquents. They are deep, very poorly drained sandy loams or loamy sands that are frequently flooded during the rainy season. A typical soil profile contains a surface layer of black sand as much as 90 cm thick overlying a gray to brown sandy subsoil down to 150 cm. The floodplain forest overstory is dominated by *Taxodium distichum, Nyssa biflora,* and *Acer rubrum.*

Upland hardwood forest soils are loamy, kaolinitic, thermic Arenic or Grossarenic Kandiudults (Lucy and Troup series, respectively). Soils are deep, well-drained to somewhat excessively well-drained, and moderately permeable sands or loamy sands found on 10-15%

slopes. The upland hardwood sites contain mixed oak-hickory forests dominated by *Quercus alba, Q. velutina, Q. falcata,* and *Carya tomentosa*.

Soils from abandoned agricultural fields (oldfields) are: loamy, kaolinitic, thermic Grossarenic Kandiudults (Troup series); loamy, siliceous, semiactive, thermic Grossarenic Paleudults (Blanton series); and loamy, kaolinitic, thermic Arenic Plinthic Kandiudults (Fuquay series). These sands or sandy loams are deep, somewhat excessively drained to moderately well drained with moderate to low permeability. A plow layer is apparent at approximately 15-20 cm depth as a result of cultivation and fertilization prior to abandonment in the 1950s. The oldfield vegetation is largely herbaceous and is dominated by various grasses (*Andropogon* spp., *Cynodon* spp.).

Longleaf pine/scrub oak forest (sandhills) soils are thermic, coated Typic Quartzipsamments (Lakeland series). These soils have sand texture to 2 m, and are very deep, excessively drained, rapidly permeable soils. Overstory vegetation is dominated by *Pinus palustris, Quercus laevis, Q. incana,* and *Q. margaretta*.

Field sampling and sample analyses

At each of the three sites per ecosystem type, a 20m x 20m plot containing three randomly distributed circular (2 m diameter) subplots was established. Twelve points were equally spaced around the circumference of each subplot, and one point was randomly selected at each sampling event to provide forest floor and mineral soil samples. Although only eight points were sampled over the course of the study, twelve points were set up to account for possible soil obstructions (*i.e.* large tree roots) that may have prevented sample collection in a given sampling event.

At each sampling point, a known area of forest floor was carefully cut and removed, and exposed mineral soil was sampled using a 2 cm-diameter coring device. Three adjacent cores were removed intact from each sampling point to 20 cm depth and partitioned into 0-5 and 5-20 cm increments. The exposed area of each soil core was gently scraped to remove any surface soil or organic material adhering along the vertical core surfaces. Samples from all three subplots were then composited by site for litter layer and mineral soils (0-5 cm depth, 5-20 cm depth). Forest floor and soil samples were placed on ice, transported to the laboratory, and refrigerated at 4°C until preparation for analysis, usually within 24 hours of sampling.

Forest floor and mineral soil samples were thoroughly homogenized and weighed out in duplicate for determination of gravimetric moisture content and pH. Moisture was determined after drying at 50°C for 72 hours, and pH values were measured with a combination glass electrode using a soil:deionized water ratio of 1:3. Subsamples of dried litter and mineral soils were ground to a fine powder using a cryogrinder and analyzed for total C and total N using a dry combustion elemental analyzer (CE2500, CE Instruments).

Duplicate soil samples were also weighed out for the determination of soluble inorganic and organic N concentrations. Soluble inorganic N concentrations were determined from 2M KCl extracts (soil:extract ratio of 1:5) using continuous flow colorimetry at the Stable Isotope/Soil Biology Laboratory (Institute of Ecology, University of Georgia). Ammonium concentrations were determined using the phenol-hypochlorite assay (U.S. Environmental Protection Agency [EPA] method 353.2) and nitrate concentrations were determined using the cadmium reduction assay (EPA method 350.1). The precision of ammonium and nitrate measurements was $<\pm 2\%$ for both analytes, and all data were corrected using known reference values in each analytical run.

Soluble organic N concentrations (SON) were determined from deionized water extracts of fresh litter (litter:deionized water ratio of 1:3) and mineral soils (soil: deionized water ratio of 1:1). Total soluble N was determined colorimetrically as total ninhydrin-reactive N (free amino-N + ammonium) using a modified ninhydrin method (Stevenson 1996). Triplicate analyses were run for duplicate extracts, and 10% of the samples were spiked with a known amount of ammonium-N (~50-100% of sample value) to check for recovery efficiency. Recovery efficiencies ranged from 93-105%. The precision of ninhydrin-N analyses were $<\pm 2\%$, and data were corrected to known reference values in each analytical run. Soil SON concentration was calculated as the difference between soil concentrations of total ninhydrin-reactive N and ammonium-N.

Although different extractants were used to remove soluble N compounds from soils in this study, Murphy *et al.* (2000) reported that concentrations of soluble mineral N and organic N did not differ in non-clay soils extracted with either 2M KCl or water. Previous trials with 2M KCl and water extracts of soils sampled from sites used in this study confirmed these findings (data not shown).

Statistical analyses

For each sampling event (n=8), values of measured soil variables were averaged by ecosystem type (n=3 per ecosystem type) for litter layer, 0-5 cm mineral soil, and 5-20 cm mineral soil. The relative distributions of SON, ammonium-N, and nitrate-N concentrations were also calculated by depth for all ecosystems at each sampling event. Because SON concentrations were not detected at all sampling events, median values were calculated to assess seasonal SON availability over the entire study.

Ecosystem-level relationships between soluble N concentrations and other measured soil variables over the study period were evaluated with linear regressions using seasonal average values (n=8 per ecosystem). Seasonal averages were also used to compare SON, ammonium-N, and nitrate-N concentrations between litter layer and mineral soils for each ecosystem type during the study. To examine patterns in soluble organic and inorganic N availability on a longer time scale and at a regional level, all seasonal soil means were averaged by depth over the two-year study and compared over all ecosystem types using linear regressions (total n=12).

Significant differences in total soluble N concentrations between sampling events were assessed for each ecosystem type by depth using seasonal averages (n=3 per type). For each ecosystem over all sampling dates, seasonal average SON, ammonium-N, and nitrate-N concentrations were compared by depth. Differences in SON, ammonium-N, and nitrate-N concentrations between ecosystem types were also evaluated by depth using two-year average seasonal values. To avoid violating assumptions of normality and homoscedasticity, all statistical tests were one-way, nonparametric analyses of variance (Kruskal-Wallis tests). Values were grouped using nonparametric Tukey-type multiple comparisons. For groups of equal sample size, the nonparametric Nemenyi multiple comparison test was used, and Dunn's nonparametric multiple comparison test was used for groups of different sample sizes or for groups with tied ranks (Zar 1996). All regressions and statistical tests were considered significant at the α=0.1 level, and concentrations are reported on a soil dry mass basis (average ± one standard error) unless otherwise noted.

RESULTS AND DISCUSSION

Bottomland hardwood forest (floodplain) soils

For each sampling event, litter layer mass and total soluble N concentrations were highly variable among the three floodplain sites (Fig. 4.3). The high level of variability at each sampling event was primarily due to measurements from one floodplain site that differed from the other two. The variant site, however, was not the same for all soluble N forms and depended on soil layer (litter layer, 0-5 cm mineral soil, 5-20 cm mineral soil).

Ecosystem variability in litter layer biomass was particularly large in Sp02, Su02, and Sp03, corresponding to one site that had a much higher abundance of *Sphagnum* moss in the forest floor. This site also contributed to the large variation in the Sp02 SON peak in 0-5 cm mineral soils. Specifically, the Sp02 increases in SON observed at the other two sites were absent from the *Sphagnum* site in the surface mineral soil, although all three sites exhibited spring flushes of SON in the 5-20 cm soil. In arctic ecosystems, *Sphagnum* relies heavily on SON for growth and uses SON preferentially over inorganic N (Kielland 1997). Similarly, the *Sphagnum* present in this study may have immobilized possible pulses of SON from the surface mineral soils during the spring, and is consistent with the observed increases in litter layer biomass (and higher site variability of the same) at those sampling events. The absence of the Sp02 SON peak in the surface mineral soil but its presence in deeper soils provides further indirect support that *Sphagnum* on the forest floor was taking up SON from surface mineral layers.

Overall patterns in ammonium-N concentrations were similar both temporally and throughout the soil profile at all floodplain sites, and were lower during the first year of sampling. Patterns in soluble N concentrations appeared closely correlated with rainfall patterns

during each sampling event. The average Su01 rainfall was similar to the expected average rainfall (97%), and total soluble N concentrations were uniformly low for all sites at all soil depths, with nitrate-N comprising >50% of the total soluble N present in the mineral soils. Although precipitation in F01, W02, however, was only ~40-60% of the expected rainfall values, increases of total soluble N concentrations in the litter layer corresponded to an expected pulse of nutrient inputs from newly fallen autumn litter (Qualls *et al.* 2002). The very soluble organic substances in the new litter were also the likely source for mineralization and nitrification transformations, leading to the concomitant increase in ammonium-N and nitrate-N concentrations in the litter layer. The sudden return of expected rainfall amounts in Sp02 likely stimulated microbial mineralization and subsequent nitrification rates in the litter layer, as supported by the significant positive correlation observed between SON and nitrate-N (Table 4.3). The Sp02 rainfall also likely leached SON from the litter layer into the mineral soils, providing a large pool of easily mineralizable N and C for microbial activity stimulated by increased water availability in all soil layers.

The effect of the Sp02 flush of SON into the mineral soil persisted into Su02, potentially increasing mineralization (ammonification) of SON and subsequent nitrification in floodplain mineral soils. The Su02 peaks and high variability of nitrate concentrations in the 0-5 cm and 5-20 cm mineral soils, however, originated from one floodplain site that was geographically isolated from the other two (Fig. 4.1). Because nitrification is an aerobic N transformation, and the continuous above average precipitation following the Su02 season likely led to increasing soil saturation that inhibited nitrification and possibly increased nitrate losses via denitrification at the distant floodplain site. Mineralization of organic matter is not as sensitive to oxygen

limitations, so coupled with high rainfall and low nitrifying activity, ammonium-N became the dominant N form for the remainder of the study at all sites.

Upland hardwood forest (hardwood) soils

For each sampling event, litter layer mass and total soluble N concentrations were similar among the three hardwood sites (Fig. 4.4). Compared to the other ecosystem types, the locations of the three hardwood sites were closer to one another on the landscape (Fig. 4.2), possibly contributing to the lower variability in measured values at each sampling event. Less variability among the hardwood sites likely allowed the detection of differences between sampling events that were obscured by higher site variability in other ecosystem types. The variability that was present at each sampling event, however, was most often due to the hardwood site that was located further away from the other two.

Hardwood litter layer mass significantly increased over the two-year study, and temporal differences in litter layer mass were reflected in the fluctuations of total soluble N concentrations in the litter layer and mineral soils. Similar to the floodplain ecosystem, temporal fluctuations in soluble N concentrations during the first year appeared related to autumn senescence and rainfall patterns. New litter inputs in F01 likely increased SON concentrations in the litter layer, which is indirectly supported by the significant positive correlation observed between litter C:N ratio and SON concentration (Table 4.3).

Carbon-to-nitrogen ratios in fresh litter are typically very high, reflecting the lack of significant decomposition of those materials (Murphy *et al.* 2000). As decomposition progresses and litter is incorporated into the mineral soil, the more easily mineralizable C and N compounds such as SON are released and consumed, leaving behind more recalcitrant organic matter that is

lower in C:N ratio (Bosatta and Ågren 1997). The significant negative relationship observed between SON and soil C:N in the hardwood mineral soils likely reflects this process (Table 4.3).

In the mineral soils, increases in SON may have derived from the fall senescence of roots and/or from SON leached from the litter layer. While we did not quantify root turnover, the possibility of leachate-derived SON seems unlikely because of the below average precipitation inputs during F01. Most of the rainfall inputs were probably absorbed in the thick hardwood litter layer where increases in total soluble N concentrations were observed, minimizing the downward transport of litter leachate into the mineral soils. This is supported by the significantly negative correlations observed between SON concentration and soil moisture in hardwood mineral soils (Table 4.3).

Absorption of rainfall in the litter layer also likely induced moisture limitations that inhibited microbial mineralization of litter SON inputs. By W02, the pulse of SON was no longer present, and the proportion of the total soluble N comprised of SON was supplanted by ammonium-N. Mineral soil layers continued to show little change until the next influx of litter in F02 that also corresponded to above average rainfall inputs. As a result, greater availability of mineralizable organic matter coupled with adequate moisture conditions resulted in significantly higher total soluble N concentrations in the litter layer that likely leached into the mineral soils.

Abandoned agricultural field (oldfield) soils

For each sampling event, litter layer mass and total soluble N concentrations varied episodically among the three oldfield sites (Fig. 4.5). Similar to both floodplain and hardwood ecosystems, most of the variability present at each sampling event was due to one site. Most of

the variability observed in the oldfield ecosystem was derived from the same site for all measurements, and this site was geographically separated relative to the other two.

There were no significant differences in litter layer mass between sampling events, although temporal fluctuations in soluble N concentrations over the entire study appeared related to autumn senescence and rainfall patterns. During F01, concentrations of SON increased in the litter layer and mineral soils, presumably because of fresh litter inputs. Similar to the hardwood ecosystem, initial influxes of litter SON at F01 likely stimulated mineralization and nitrification rates in the litter layer and resulted in the appearance of nitrate-N. Unlike hardwood ecosystems, infiltration of rainfall inputs to the surface mineral soil was more likely since the oldfield litter layer was thinner and less dense, resulting in the detection of nitrate-N. Percolation of litter leachate through the vertical soil profile, however, was probably limited by the plow-layer present in the 5-20 cm soil layer and is supported by the significant negative correlation observed between SON and soil moisture content (Table 4.3). The large increase in nitrate-N concentrations in 5-20 cm soils at W02 was observed in the distant oldfield site, possibly due to site-specific effects on water availability that were not present at the other two oldfields.

Lower litter layer mass and higher mineral soil concentrations of soluble N corresponded with the return of expected rainfall inputs during Sp02 and Su02, suggesting that increased moisture during the warmer seasons released/mobilized SON compounds from decomposing litter and stimulated microbial N transformation rates. In the litter layer, SON lost from decomposing litter likely provided a constant supply of mineralizable substrate for ammonifiers and subsequently increased nitrification rates.

In the mineral soils, however, the relationship between SON and ammonifiers were significantly negative, suggesting that the pool of SON in the mineral soils were being

mineralized faster than SON could be supplied from the litter above (Table 4.3). Although the negative relationship between C:N ratio and SON in the surface mineral soils suggests that SON was being released during the decomposition of older organic matter in the soil itself, the rates of SON supply were not sufficient to offset SON consumption in oldfield mineral soils.

Longleaf pine/scrub oak forest (sandhills) soils

For each sampling event, litter layer mass and total soluble N concentrations were highly variable among the three sandhills sites (Fig. 4.6). Most of the variability present at each sampling event was due to one site that was much older and had not experienced any recent disturbance. Of the other two sites, one was experimentally burned in the early 1980s, and the other was located in close proximity to an operational government area and had likely experienced some disturbance during SRS construction in the 1950s (?). While temporal SON fluctuations were similar for all sites, average concentrations were strongly influenced by the high values measured in the older sandhills site in the litter layer and 0-5 cm mineral soils. Similarly, inorganic N concentrations fluctuated similarly for all three sites, but average concentrations were strongly influenced by the high values of ammonium-N in the litter layer and nitrate-N in all layers from the two disturbed sandhills sites.

As a result of site variability, litter layer mass and soluble N concentrations in the sandhills ecosystem did not differ significantly between sampling events (Fig. 4.6). Temporal fluctuations, however, did appear to correspond with litterfall and rainfall inputs, similar to the other ecosystems examined in this study. Increases in SON concentrations corresponded with autumn senescence in F01 and remained high throughout W02. Similar to the hardwood ecosystem during W02, increases in the total soluble N concentrations in the litter layer and

absence of any changes in the mineral soils suggested that N transformation rates in the thick sandhills litter layer were attributable to rainfall inputs that did not reach the mineral soil. The significant negative correlation observed between SON and soil moisture in 0-5 cm soils supports this idea (Table 4.3).

Soluble organic N concentrations in the litter layer paralleled increasing litter layer mass through Sp02 when higher precipitation inputs leached SON from litter into mineral soils. Above-average rainfall inputs and warmer summer temperatures contributed to increased microbial activity that appeared to consume the flush in soluble N concentrations that had occurred in the previous season. No apparent increase in litter layer mass in F02 suggested that autumn litterfall inputs were low, as evidenced by very low SON concentrations in both litter layer and mineral soils. Increases in total soluble N during the winter months likely corresponded to lower microbial activity in colder temperatures.

Overall, SON concentrations in the litter layer showed significant positive correlation with litter layer mass (Table 4.3), and SON concentrations between litter layer and mineral soils were significantly correlated (see following section). Interestingly, seasonal soluble inorganic N concentrations between sandhills forest floor and mineral soils were negatively correlated. The negative relationship may have been related to litter quality or acidity that influenced adsorption/desorption dynamics of ammonium-N within the forest floor (Qualls *et al.* 2002). Northup *et al.* reported similar relationships for strongly acidic *Pinus muricata* forest floors and showed that increasing litter polyphenolic (*i.e.* tannin) content reduced the rate of inorganic N release while increasing the release of organic N as protein-tannin complexes (1995). Furthermore, the authors suggested that pines could directly utilize these protein-tannin complexes via ectomycorrhizal associations, effectively "monopolizing" litter N by

immobilizing it into a form not readily available to soil microorganisms. Although we cannot confirm this in the present study, the significant positive correlations observed between forest floor SON concentrations and both forest floor biomass and acidity (Table 4.3) support the idea that the negative relationship between forest floor and mineral soil ammonium-N concentrations was due to litter-specific N dynamics.

Temporal patterns of soluble N availability in the vertical soil profile

Total soluble N concentrations in the litter layer were an order of magnitude higher than mineral soil concentrations in hardwood, oldfield, and sandhills ecosystems (Table 4.2). Although this difference was not observed in the floodplain sites, concentrations of all soluble N forms decreased with increasing soil depth in all ecosystem types. All ecosystem types also exhibited significant correlations in SON concentrations between litter layer and mineral soils over all sampling events, supporting observations in this and other studies that the leaching of SON from forest floor horizons was the likely source of SON in surface mineral soils (Qualls *et al.* 1991, Huang and Schoenau 1998, Michalzik *et al.* 2001, Zhong and Makeschin 2003).

For floodplain, hardwood, and oldfield ecosystems, ammonium-N was the dominant soluble N compound and was present at all sampling events in all depths for all ecosystems. Concentrations and relative availability of ammonium-N were typically higher in the second year than the first year of this study, and appeared to be correlated with rainfall inputs that mobilized SON in the litter layer. The predominance of ammonium-N is consistent with abundant literature that ammonium-N is the major inorganic N source in many temperate ecosystem soils, particularly when the SON component is not considered. When SON is incorporated as a potentially available N compound, however, the relative availabilities of ammonium-N (and

nitrate-N) shift and, in some cases, become the minor available form(s) as in the case for sandhills soils.

Compared to ammonium-N, sandhills SON concentrations comprised an equal or greater proportion of total soluble N in most sampling events. The relative availability of SON to total soluble N in the sandhills mineral soils is similar to those found in tundra and boreal forest soils (Kielland 1995, Nasholm *et al.* 1998, Huang and Schoenau 1998). Despite the difference in temperature and other variables related to climate, both soils are highly acidic and experience conditions that limit decomposition and N mineralization, leading to the accretion of organic matter. In boreal soils, low temperatures and low pH maintain high soil organic contents throughout the soil profile (Atkin 1996) while in sandhills soils, low pH, limited water availability, and low litter quality (high secondary compound content) lead to organic matter buildup in the litter layer that leach organic compounds into the mineral soils.

Despite its dominance in sandhills soils, soluble organic N was not available at every sampling event, both in sandhills and in the other ecosystems. As a result, median values of SON concentrations and the relative proportion of total soluble N it comprised were typically very low over the entire study (Fig. 4.7). When SON was present, the proportion of total soluble N comprised by SON was highly variable but, on average, exceeded 20% for all ecosystems in both forest floor and mineral soils. The episodic nature of SON availability was likely influenced by synergistic or antagonistic interactions of several potential controls (*i.e.* temperature, pH, moisture, litter quality) in the field (Michalzik *et al.* 2001).

Nitrate-N was also not present in all soils at all sampling events and was not significantly different from SON in all ecosystems except the sandhills (Figs. 4.3-4.6). Of the soluble N compounds measured, nitrate-N consistently comprised the smallest proportion of soil total

soluble N concentrations for all sites. Similar to SON dynamics, the availability of nitrate-N was highly variable between sampling events, but constituted ~10-40% of total soluble N concentrations when present.

Landscape patterns of soluble N availability in relation to soil characteristics

At the ecosystem scale, temporal relationships between soluble organic and inorganic concentrations with other measured soil properties varied by ecosystem type and by soil depth (Table 4.3). By averaging values from each sampling event over the entire study period, however, temporal variations were removed. As a result, relationships between soil physicochemical properties and soluble N concentrations that were not apparent at the ecosystem scale emerged at the landscape scale when data from all depths in all ecosystem types were compared (Figs. 4.8, 4.9). Overall, soluble N availability averaged over the entire study corresponded to ecosystem characteristics that were strongly influenced by broad environmental gradients (Fig. 4.10). This supports observations from other studies that factors controlling N dynamics change with both temporal and spatial scales (Michalzik *et al.* 2001).

Overall, two-year average seasonal concentrations of SON and soluble inorganic N (ammonium-N + nitrate-N) were positively correlated with moisture content, total C and total N, and were negatively correlated with pH, soil temperature (at 5 cm), and percent sand (Figs. 4.7, 4.8). Specifically, both SON and soluble inorganic N (ammonium-N + nitrate-N) concentrations exhibited similar positive and negative relationships with the same soil factors, but slopes for soluble inorganic N were always steeper than SON slopes in all regressions. The supply of soil inorganic N is largely mediated by microbial processes, and although decomposition of organic matter also contributes to the supply of SON in soils, the majority of the SON pool is derived

from the physical desorption of SON from organic matter in the litter layer (Qualls *et al.* 2001). Based on our data, it appeared that the biological mechanisms controlling inorganic N concentrations were more sensitive to changing soil conditions compared to the primarily physicochemical mechanisms controlling SON concentrations in the ecosystems examined. Although the sensitivity of response to soil changes differed between these mechanisms, the similar direction of responses likely contributed to the significant positive correlation observed between soluble organic and inorganic N across all ecosystems in both forest floor and mineral soils (Fig. 4.8).

While there were significant individual correlative relationships between soil physicochemical properties and N concentrations, the mechanisms driving the correlations were probably influenced by interactions between the soil properties themselves. In this study, all ecosystem soils were coarse-textured, so the effect of sand content on soil moisture and drainage likely played an important role in soil N transformations in the mineral soil. Specifically, the moisture holding capacity of coarse-textured soils is lower than finer-textured soils because of less surface area contact with water. As a result, water and the dissolved nutrients in solution are easily leached through the soil profile (Appel and Mengel 1993). In addition, SON is more likely to be retained in soils with higher clay content because of the adsorption/desorption dynamics of organic compounds from fine particles. These general relationships corresponded to the lower soluble N concentrations in sandhills and oldfield soils that were coarser-textured and lower in both organic matter and moisture content compared to the mesic, more organic hardwood and floodplain ecosystem soils (Tables 4.1, 4.2).

CONCLUSIONS

Overall, seasonal concentrations of SON, ammonium-N, and nitrate-N were highly variable during this two-year study in both forest floor and mineral soils of all ecosystem types and were strongly related to rainfall events. Concentrations of soluble N were greatest in forest floors and decreased with soil depth, and litter dynamics controlling the release of N from forest floors strongly influenced soluble N concentrations in the mineral soils of all ecosystems.

While seasonal relationships were not immediately apparent at the individual ecosystem scale, relationships between both soluble organic and inorganic N and with other soil variables averaged over the entire study revealed the influence of broad environmental gradients on soluble N availability. Our results support published studies showing that patterns in soluble N availability shift with changing spatial and temporal scales, and that SON likely plays a key role in the N dynamics of forest floors and surface mineral soils in these temperate Upper Coastal Plain ecosystems.

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Figure 4.1. Map of ecosystem sites sampled July 2001 through April 2003 in the U.S. Department of Energy's Savannah River Site (SRS). *F* bottomland hardwood forest (floodplain), *H* upland hardwood forest (hardwood), *O* abandoned agricultural field (oldfield), *S* longleaf pine/scrub oak forest (sandhills). Sites are identified as such throughout this study.

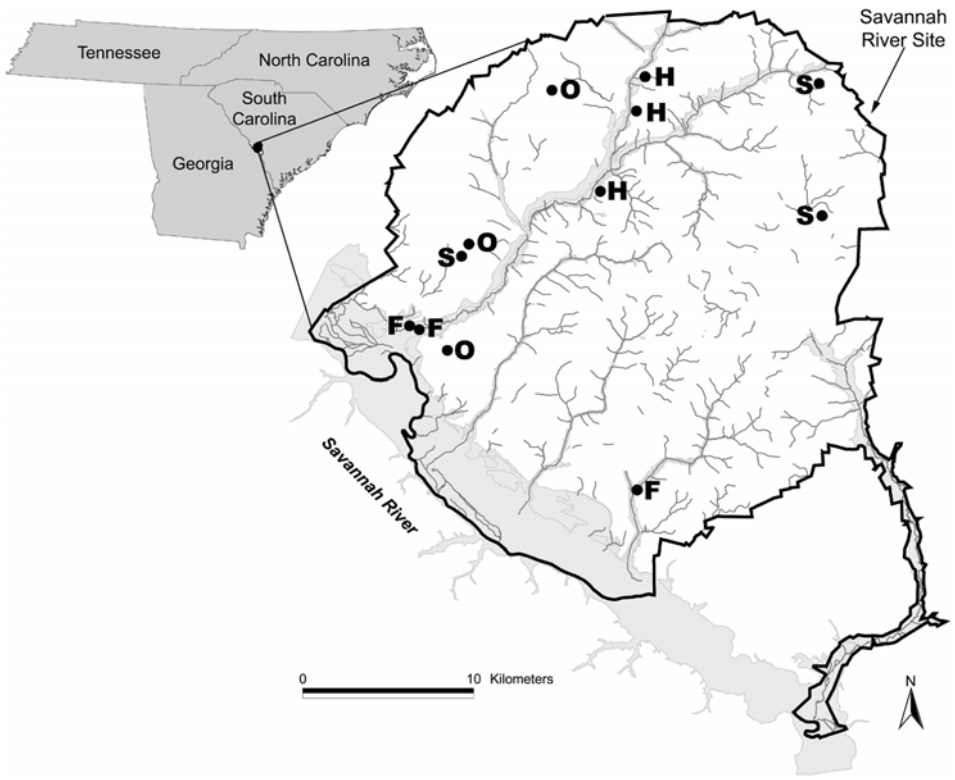

Tennessee
North Carolina
South Carolina
Georgia
Savannah River Site
Savannah River
0
10 Kilometers
N

Figure 4.2. Monthly precipitation values for months when sampling events occurred. Bars represent 100-year monthly average (1903-2003). Signs represent observed monthly precipitation during the study and direction of variation from the 100-year average. In this figure and Figs. 4.3-4.6, sampling dates are shown as season (*Su* summer, *F* fall, *W* winter, *Sp* spring) followed by year (*01* 2001, *02* 2002, *03* 2003).

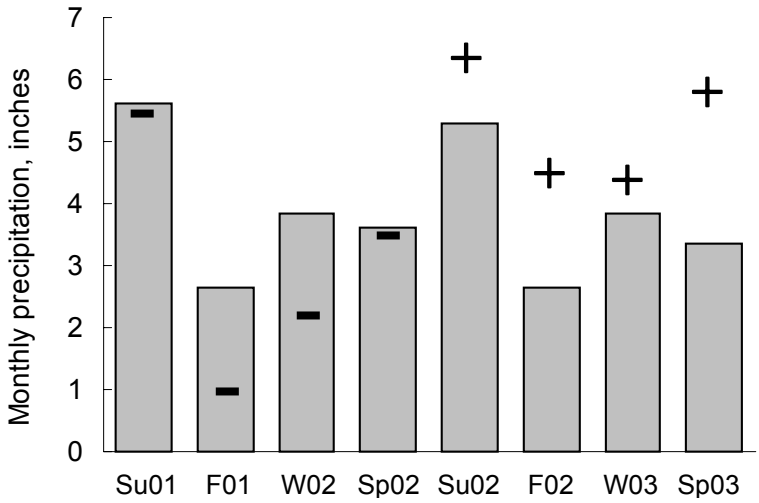

Monthly precipitation, inches
7
6
5
4
3
2
1
0
Su01
F01
W02
Sp02
Su02
F02
W03
Sp03

Figure 4.3. Seasonal averages (± one standard error, s.e.) of litter layer mass and total soluble N concentrations in floodplain soils (*n*=3). Arrows show average seasonal values over the study period. In each graph, different lower case letters indicate seasonal differences in total soluble N concentrations. Different upper case letters denote differences between SON, ammonium-N, and nitrate-N concentrations over the two-year study.

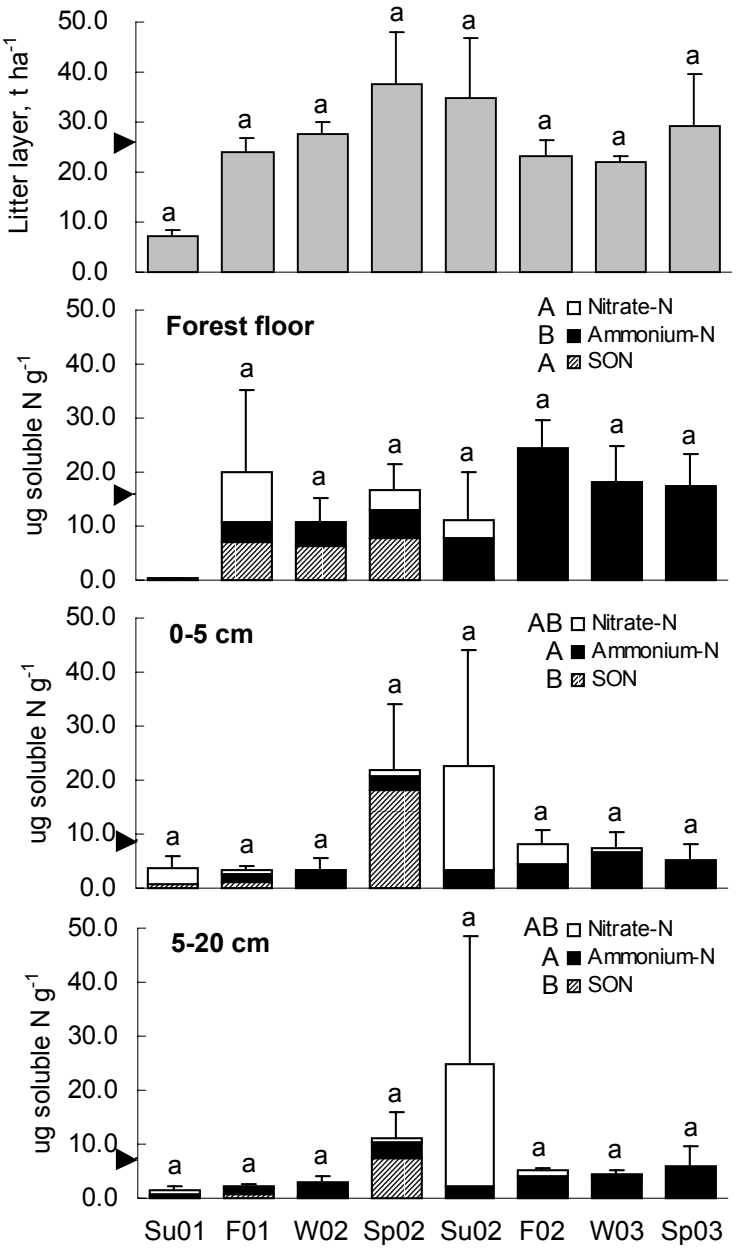

Litter layer, t ha-1
50.0
40.0
30.0
20.0
10.0
0.0
Forest floor
A Nitrate-N
B Ammonium-N
A SON
ug soluble N g-1
0-5 cm
AB Nitrate-N
A Ammonium-N
B SON
5-20 cm
Su01
F01
W02
Sp02
Su02
F02
W03
Sp03

Figure 4.4. Seasonal averages (± 1 s.e.) of litter layer mass and total soluble N concentrations in hardwood soils (*n*=3). Arrows show average seasonal values over the study period. In each graph, different lower case letters indicate seasonal differences in total soluble N concentrations. Different upper case letters denote differences between SON, ammonium-N, and nitrate-N concentrations over the two-year study.

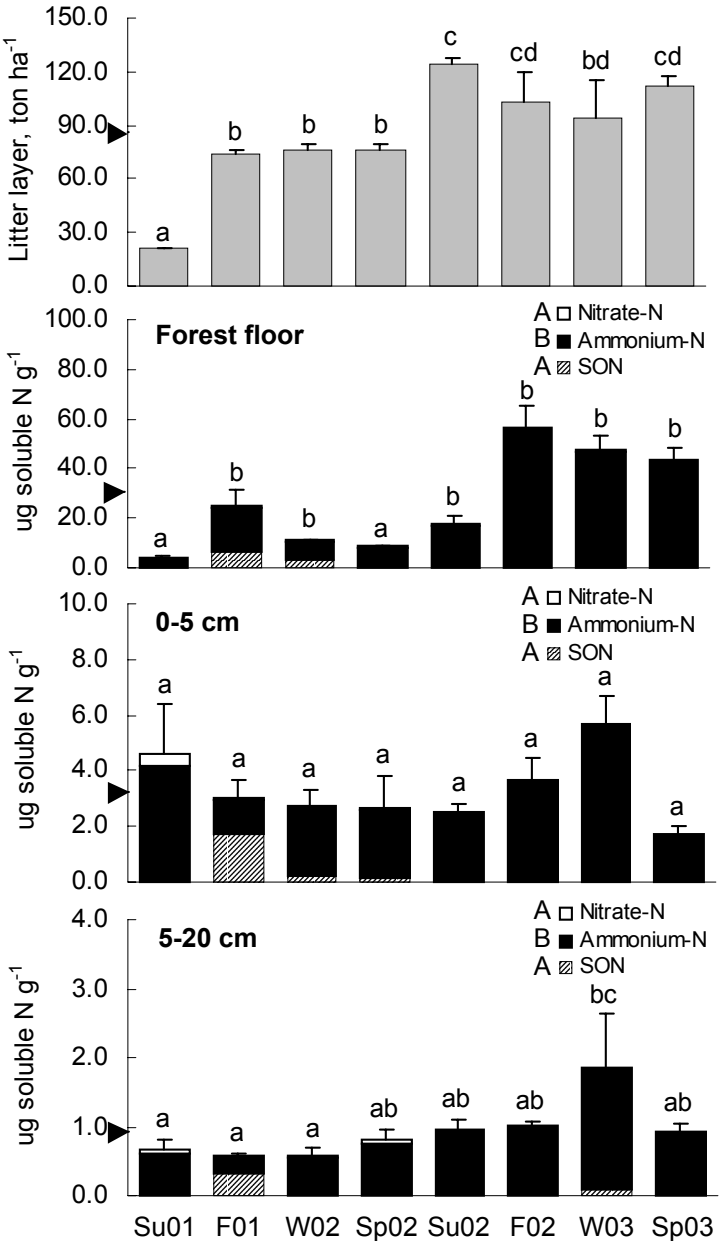

Litter layer, ton ha-1
150.0
120.0
90.0
60.0
30.0
0.0
a
b
b
b
c
cd
bd
cd
Forest floor
A Nitrate-N
B Ammonium-N
A SON
ug soluble N g-1
100.0
80.0
60.0
40.0
20.0
0.0
0-5 cm
10.0
8.0
6.0
4.0
2.0
5-20 cm
4.0
3.0
2.0
1.0
bc
ab
Su01
F01
W02
Sp02
Su02
F02
W03
Sp03

Figure 4.5. Seasonal averages (± 1 s.e.) of litter layer mass and total soluble N concentrations in oldfield soils (*n*=3). Arrows show average seasonal values over the study period. In each graph, different lower case letters indicate seasonal differences in total soluble N concentrations. Different upper case letters denote differences between SON, ammonium-N, and nitrate-N concentrations over the two-year study (*nd* no data).

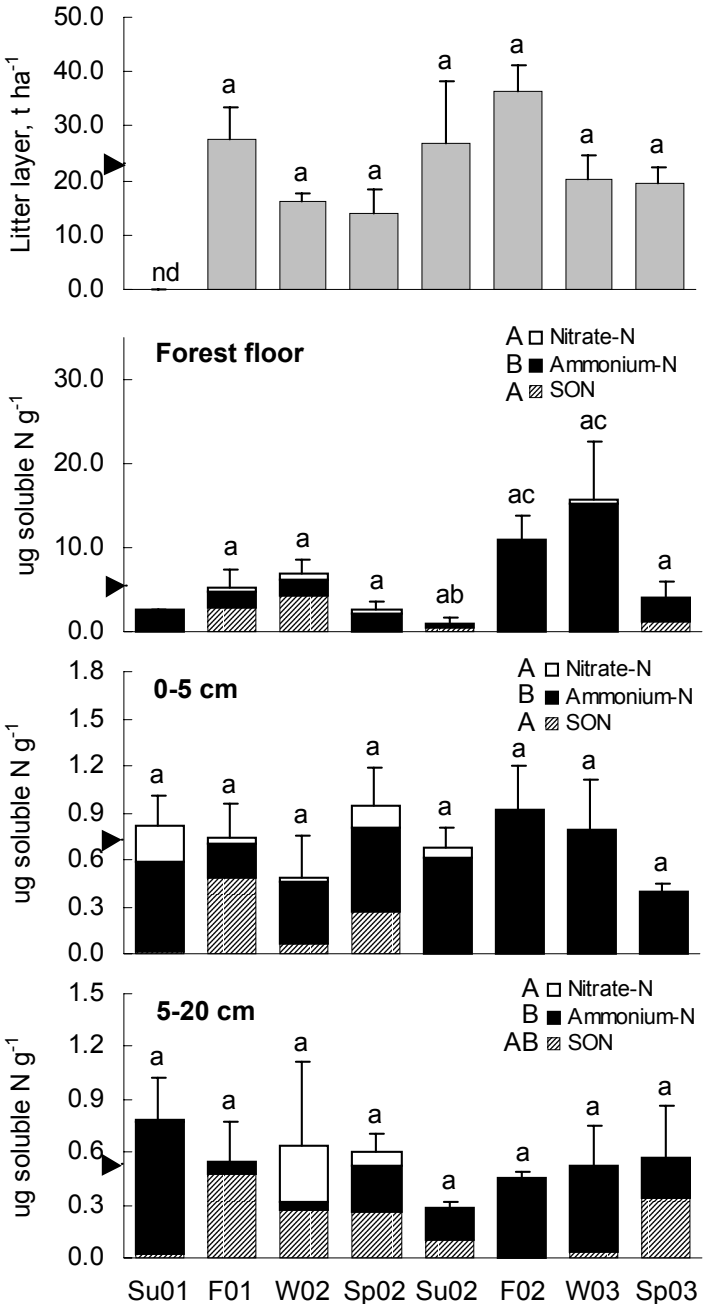

Litter layer, t ha-1
50.0
40.0
30.0
20.0
10.0
0.0
nd
a
a
a
a
a
a
a
Forest floor
A Nitrate-N
B Ammonium-N
A SON
ug soluble N g-1
30.0
20.0
10.0
0.0
a
a
a
ab
ac
ac
a
0-5 cm
A Nitrate-N
B Ammonium-N
A SON
ug soluble N g-1
1.8
1.5
1.2
0.9
0.6
0.3
0.0
a
a
a
a
a
a
a
a
5-20 cm
A Nitrate-N
B Ammonium-N
AB SON
ug soluble N g-1
1.5
1.2
0.9
0.6
0.3
0.0
a
a
a
a
a
a
a
a
Su01
F01
W02
Sp02
Su02
F02
W03
Sp03

Figure 4.6. Seasonal averages (± 1 s.e.) of litter layer mass and total soluble N concentrations in oldfield soils (*n*=3). Arrows show average seasonal values over the study period. In each graph, different lower case letters indicate seasonal differences in total soluble N concentrations. Different upper case letters denote differences between SON, ammonium-N, and nitrate-N concentrations over the two-year study.

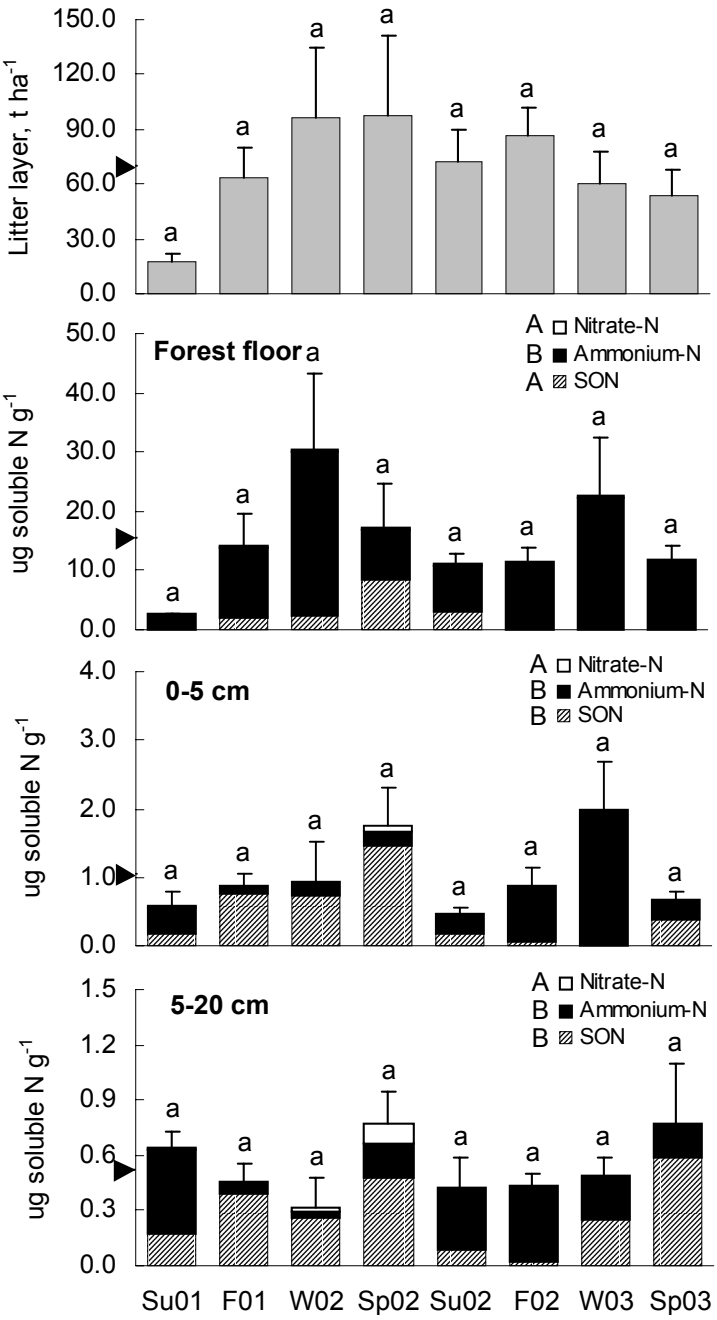

Litter layer, t ha-1
150.0
120.0
90.0
60.0
30.0
0.0
a
Forest floor
A Nitrate-N
B Ammonium-N
A SON
ug soluble N g-1
50.0
40.0
30.0
20.0
10.0
0.0
0-5 cm
B Nitrate-N
B Ammonium-N
B SON
4.0
3.0
2.0
1.0
0.0
5-20 cm
1.5
1.2
0.9
0.6
0.3
0.0
Su01
F01
W02
Sp02
Su02
F02
W03
Sp03

Figure 4.7. Median and mean values of ecosystem SON concentrations. Median values represent SON concentrations over all seasons, and mean (±s.e.) concentrations show average concentrations for seasons when SON was present. In each graph, different lower case letters indicate significant differences between ecosystem average values.

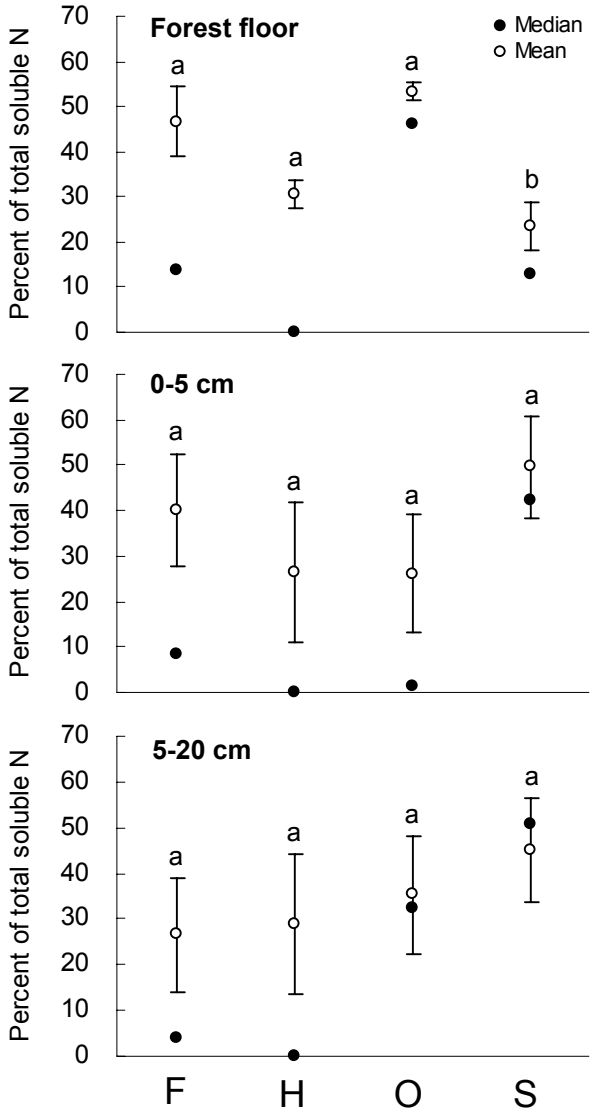

Forest floor
Median
Mean
0-5 cm
5-20 cm
Percent of total soluble N
70
60
50
40
30
20
10
0
a
a
a
b
a
a
a
a
a
a
a
a
F
H
O
S

Figure 4.8. Relationships between soluble organic (inorganic) N concentrations and selected physical soil variables for all ecosystems in forest floor and mineral soils. Solid regression line and markers represent SON, and dotted regression line and hollow markers represent soluble inorganic N. ◆Floodplain, ■Hardwood, ▲Oldfield, ●Sandhills.

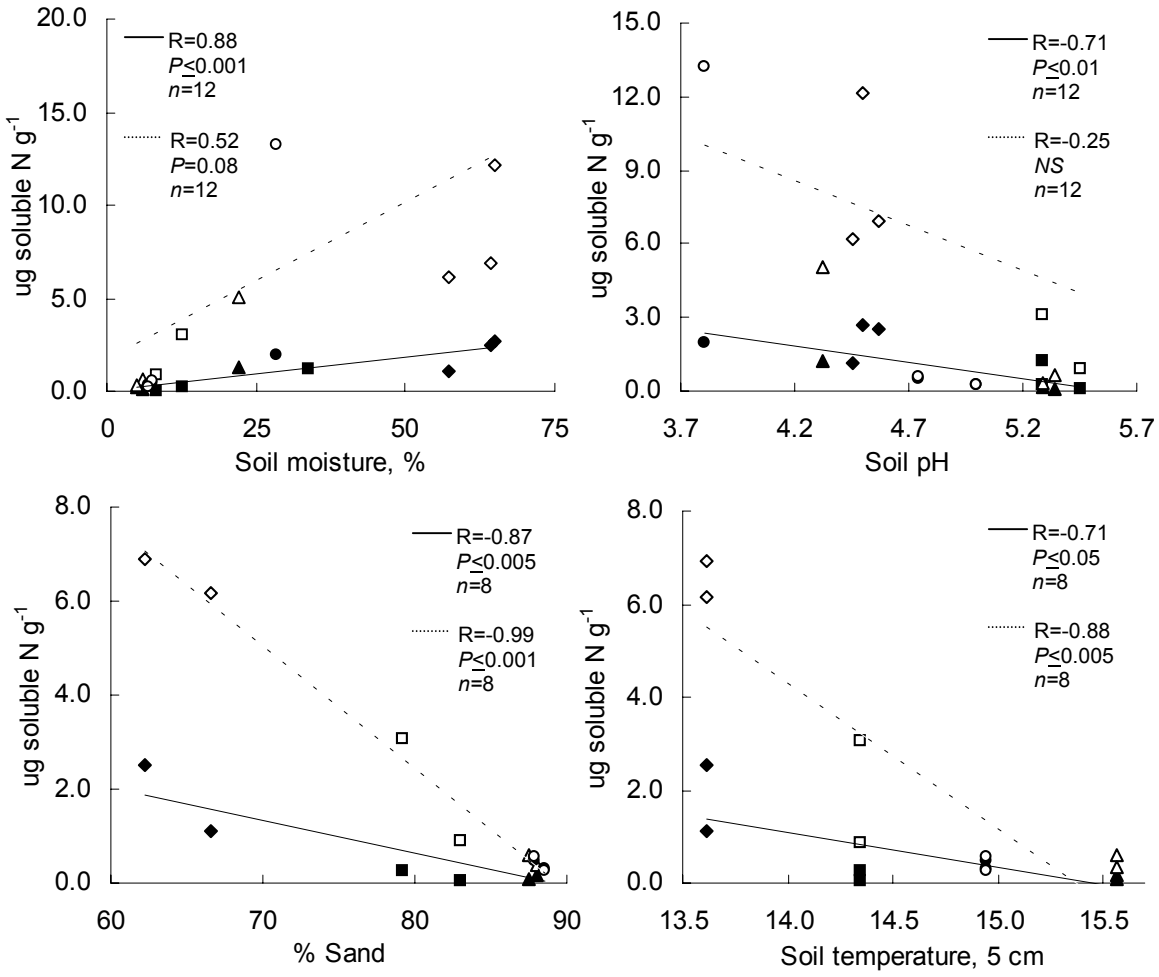
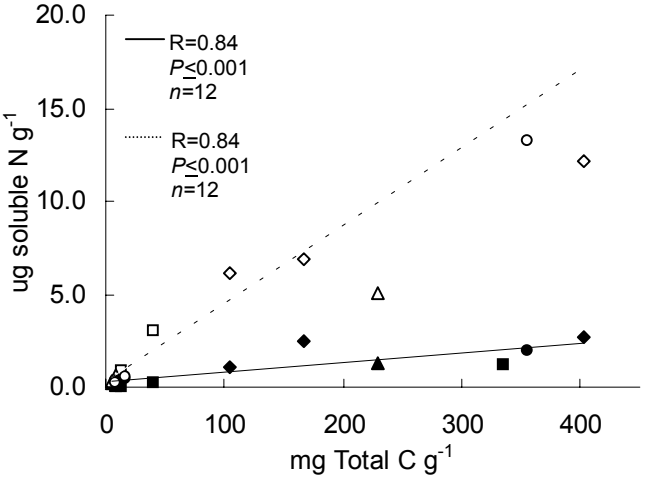
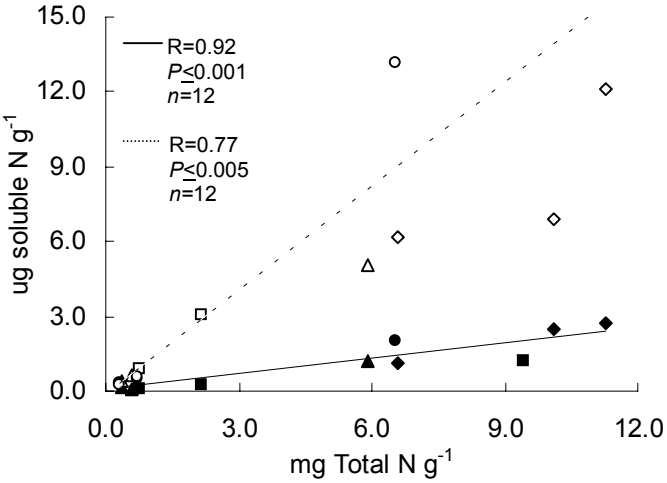

ug soluble N g-1
R=0.88
P≤0.001
n=12
R=0.52
P=0.08
n=12
Soil moisture, %
R=-0.71
P≤0.01
n=12
R=-0.25
NS
n=12
Soil pH
R=-0.87
P≤0.005
n=8
R=-0.99
P≤0.001
n=8
% Sand
R=-0.71
P≤0.05
n=8
R=-0.88
P≤0.005
n=8
Soil temperature, 5 cm

Figure 4.9. Relationships between soluble organic and inorganic N concentrations, and correlation of each with total carbon and nitrogen for all ecosystems in forest floor and mineral soils. Solid regression line and markers represent SON, and dotted regression line and hollow markers represent soluble inorganic N. Symbol legend in Fig. 7.


20.0
15.0
10.0
5.0
0.0
ug soluble N g-1
R=0.84
P≤0.001
n=12
R=0.84
P≤0.001
n=12
0
100
200
300
400
mg Total C g-1


15.0
12.0
9.0
6.0
3.0
0.0
ug soluble N g-1
R=0.92
P≤0.001
n=12
R=0.77
P≤0.005
n=12
0.0
3.0
6.0
9.0
12.0
mg Total N g-1

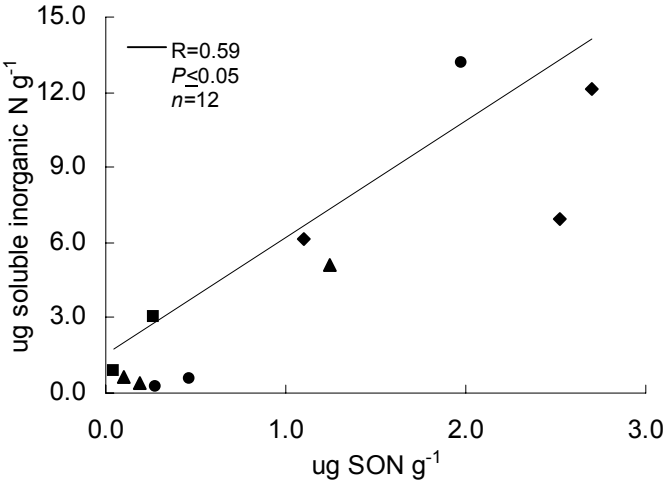
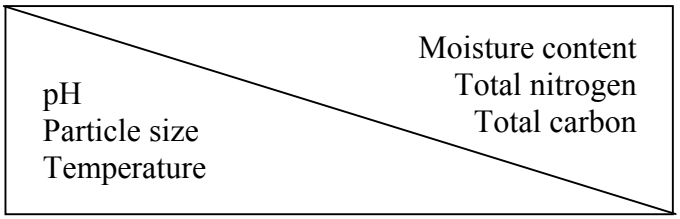

15.0
12.0
9.0
6.0
3.0
0.0
ug soluble inorganic N g-1
R=0.59
P≤0.05
n=12
0.0
1.0
2.0
3.0
ug SON g-1

Figure 4.10. Schematic representation of physical and chemical factors influencing soluble N concentrations in temperate Upper Coastal Plain ecosystems. The larger area of each triangle represents increasing values for variables identified in each.



LOW ——Total soluble N concentration——▶ **HIGH**

Table 4.1. Ranges in seasonal values of selected soil properties for ecosystem types sampled over two years (*n*=3 per ecosystem type). Values in parentheses are averages of each measurement over the course of this study. Forest floor is identified as FF, and mineral soils as 0-5 cm and 5-20 cm. In this table and Tables 2 and 3, *F* floodplain, *H* hardwood, *O* oldfield, *S* sandhills.

Type	Depth	SM	Sand	pH	Total C	Total N
	--cm--	--% mass--	----%----	------1:3-----	----------mg g^{-1}----------	
F	FF	56-79 (65)	.	4.2-4.7 (4.5)	338-465 (403)	10.8-11.9 (11.3)
	0-5	51-74 (64)	53-74 (62)	3.9-5.1 (4.6)	141-199 (167)	8.4-12.5 (10.1)
	5-20	48-69 (57)	31-86 (67)	3.9-4.9 (4.5)	62-135 (105)	3.4-8.3 (6.6)
H	FF	23-46 (34)	.	5.0-5.8 (5.3)	218-390 (335)	6.8-11.3 (9.4)
	0-5	8-16 (13)	76-82 (79)	4.9-5.7 (5.3)	27.0-50.1 (39.7)	1.2-2.7 (2.2)
	5-20	6-10 (8)	81-85 (83)	5.1-5.6 (5.5)	8.9-21.3 (13.8)	0.5-1.1 (0.7)
O	FF	3-35 (22)	.	4.1-4.6 (4.3)	150-302 (229)	4.1-8.5 (5.9)
	0-5	2-12 (6)	84-91 (88)	5.0-5.7 (5.3)	5.9-9.7 (8.1)	0.4-0.8 (0.6)
	5-20	3-6 (5)	84-91 (88)	4.9-5.6 (5.3)	2.6-12.8 (5.5)	0.2-0.9 (0.4)
S	FF	17-41 (28)	.	3.6-4.0 (3.8)	260-429 (356)	4.4-7.5 (6.6)
	0-5	5-14 (8)	84-91 (88)	4.4-5.3 (4.7)	13.3-26.9 (17.6)	0.6-0.8 (0.7)
	5-20	5-10 (7)	87-90 (89)	4.7-5.6 (5.0)	5.8-10.6 (8.0)	0.2-0.6 (0.3)

Table 4.2. Correlation coefficients between seasonal average soluble organic N (μg N g^{-1}) and other measured soil variables. Values for forest floor are identified as FF, and mineral soils as 0-5 cm and 5-20 cm. FF_m forest floor biomass (tons ha^{-1}), *T* soil temperature at 5 cm, *SM* percent soil moisture, pH_w 1:3 water:fresh weight ratio. $^*P \leq 0.1$, $^{**}P \leq 0.05$, $^{***}P \leq 0.01$.

Type	Depth	FF_m	T	SM	pH_w	C	N	C:N	NH_4^+-N	NO_3^-N
	--cm--	t ha^{-1}	°C	%	1:3	---mg g^{-1}---			------μg N g^{-1}-------	
F	FF	0.40	-0.20	-0.52	-0.36	0.08	-0.21	0.19	-0.56	0.63*
	0-5	0.49	-0.08	0.15	0.44	0.42	0.72**	-0.37	-0.23	-0.17
	5-20	0.50	-0.09	-0.07	0.28	0.01	0.19	-0.44	-0.10	-0.16
H	FF	-0.20	-0.20	-0.48	-0.27	0.28	-0.24	0.58*	-0.32	0.55
	0-5	-0.18	-0.01	-0.71**	-0.12	-0.12	0.19	-0.51	-0.53	-0.19
	5-20	-0.12	-0.13	-0.62*	-0.26	-0.30	0.32	-0.68*	-0.33	-0.34
O	FF	-0.25	-0.29	0.19	0.16	-0.10	-0.19	0.20	-0.48	0.66*
	0-5	-0.07	0.02	0.18	0.42	-0.17	0.52	-0.73**	-0.63*	0.07
	5-20	-0.37	0.13	-0.72**	0.11	-0.34	-0.18	-0.15	-0.77**	0.24
S	FF	0.58*	0.48	-0.39	-0.84***	-0.01	-0.11	0.01	-0.13	0.10
	0-5	0.50	0.29	-0.72**	0.67*	-0.68*	-0.47	-0.50	-0.56	0.74**
	5-20	0.02	0.16	0.26	0.24	0.25	0.18	0.08	-0.62*	0.40

Table 4.3. Two-year averages of seasonal litter layer mass (±1 s.e) and concentrations (relative proportions) of soluble N (*n*=8 sampling events) by depth in all ecosystems (*n*=3 per ecosystem). Values in parentheses correspond to percent of the total soluble N comprised by soluble N form indicated. For each forest floor and mineral soils, significant differences between ecosystem soluble N concentrations (relative proportions) are indicated by different lower case (upper case) letters.

Depth	Type	Litter layer	SON	Ammonium-N	Nitrate-N
		---ton ha^{-1}---	--μg N g^{-1} (%)--	--μg N g^{-1} (%)--	--μg N g^{-1} (%)--
FF	F	25.7±3.3 a	2.70 a (23 A)	10.04 ab (64AB)	2.08 a (13A)
	H	85.0±11.2 b	1.23 a (8 A)	25.26 a (91 A)	0.27 a (2 A)
	O	23.0±2.7 a	1.24 a (29 A)	4.75 b (63 B)	0.32 a (7 A)
	S	68.5±9.2 b	1.98 a (15 A)	13.06 ab (85AB)	0.13 a (1 A)
0-5 cm	F	.	2.52 a (20 A)	3.36 a (57BC)	3.55 a (21 A)
	H	.	0.27 ab (10B)	2.99 ab (89 A)	0.07 ab(2BC)
	O	.	0.11 b (13AB)	0.55 abc(78 C)	0.06 ab (7AC)
	S	.	0.47 ab (43A)	0.53 c (54BC)	0.02 b (2 C)
5-20 cm	F	.	1.11 a (20 B)	2.88 a (61AC)	3.28 a (19 A)
	H	.	0.05 b (7 B)	0.86 a (90BC)	0.02 a (3AB)
	O	.	0.19 ab (31AB)	0.31 a (65 A)	0.05 a (4 B)
	S	.	0.28 ab (45 A)	0.24 a (49BC)	0.02 a (2 B)

CHAPTER 5

SOIL AVAILABILITY AND PLANT UPTAKE OF SOLUBLE MINERAL AND ORGANIC NITROGEN IN TEMPERATE FLOODPLAIN AND SANDHILLS SOILS[1]

[1]Jin, V.L., C.S. Romanek, R.R. Sharitz, and L.A. Donovan. To be submitted to *Ecology Letters*.

ABSTRACT

Amino compounds constitute part of the soluble organic N (SON) pool in soils that is easily mineralizable, and so play important roles in N transformations and, subsequently, plant nutrition. To assess the relationship between N availability and *in situ* plant uptake rates of organic and mineral N, soil N availability was quantified as soil soluble N concentrations and gross mineralization rates. In addition, plant uptake of stable isotope-labeled ^{15}N-[2]-^{13}C-glycine and ^{15}N-ammonium sulfate was measured in two temperate tree species (*Acer rubrum* L., *Pinus palustris* Miller) in floodplain and sandhills sites of the southeastern USA. Soluble organic N (SON, free amino-N) concentrations equaled or exceeded inorganic N concentrations at both sites, but SON and ammonium-N were significantly higher in floodplain (11.6±1.1 μg SON g^{-1}, 9.0± 1.2 μg NH_4^+-N g^{-1}) than sandhills (3.6±0.3 μg SON g^{-1}, 1.3± 0.2 μg NH_4^+-N g^{-1}) soils. There was no difference in nitrate-N concentrations between floodplain (0.10±0.01 μg NO_3^--N g^{-1}) and sandhills (0.52±0.27 μg NO_3^--N g^{-1}) soils. Gross mineralization rates were significantly higher in sandhills soils (m_S: 3.7±0.3 μg N g^{-1} d^{-1}) than on floodplain soils (m_F: 0.7±1.8 μg N g^{-1} d^{-1}). Relationships between soil N availability and plant N uptake were variable and differed over time. Roots of *A. rubrum* and *P. palustris* competed well against soil N immobilization factors for available ammonium-N in floodplain and sandhills soils, respectively. Although the uptake of intact glycine was not apparent in plant roots at either site, glycine additions influenced soil N dynamics and plant N nutrition by providing easily mineralizable N and C in these two contrasting temperate ecosystems.

KEYWORDS: glycine, plant organic N uptake, soluble organic nitrogen, *Pinus palustris, Acer rubrum*

INTRODUCTION

Primary productivity in many terrestrial ecosystems is often limited by nitrogen (N) availability (Vitousek *et al*. 1997), so plant uptake of soluble organic N (SON) compounds in addition to soluble inorganic N compounds (ammonium, NH_4^+; nitrate, NO_3^-) is potentially important in plant N nutrition and ecosystem N cycling (Schimel and Chapin 1996, Lipson and Näsholm 2001). Root uptake of SON as amino acids is documented in both mycorrhizal and non-mycorrhizal plants (Kielland 1994, Raab *et al*. 1999), most notably in arctic, alpine, and boreal ecosystems where soil N mineralization processes are limited due to low temperatures and low pH (Atkin 1996). Amino acid uptake by plant roots, however, occurs in plant species from various other ecosystems as well, including temperate agricultural fields (Näsholm *et al* 2000, Bardgett *et al.* 2003) and subtropical heaths (Schmidt and Stewart 1997). Since soil concentrations of amino-N compounds often equal or exceed inorganic N concentrations (Schulten and Schnitzer 1998) and kinetic uptake parameters for amino acids and inorganic N can be very similar (Jones and Darrah 1994), plants could substantially increase their available nitrogen resources by using both organic and inorganic N compounds. Both intact and excised plant roots readily take up amino acids from hydroponic solutions (Persson and Näsholm 2002, Henry and Jeffries 2003a), but the ability of plant roots to compete with soil microorganisms for soil organic N in the field remains unclear (Jones 1999, Hodge *et al.* 2000, Näsholm and Persson 2001).

Similar to plant-microbial competition for soil inorganic N, plant roots are considered inferior competitors for SON compared to soil microorganisms (Owen and Jones 2001). Ostensibly, the ability of plant roots to use soil SON will depend on the same uptake processes and factors that control inorganic N uptake, including soil N concentration, N supply rate, and

root density (Leadley *et al*., 1997). While *in situ* uptake of amino acids in soil is documented in various plant species growing in cooler regions (McKane *et al.* 2002, Henry and Jeffries 2003b) where SON commonly constitutes a larger proportion of total soil N (Schulten and Schnitzer 1998), far fewer studies have examined these processes in warm temperate ecosystems. Furthermore, *in situ* uptake of soil amino acid N by plants growing in well-drained soils where mineralization processes are not as limited have not been characterized.

In this study, we used temperate bottomland hardwood forest (floodplain) and longleaf pine-scrub oak (sandhills) forest soils containing tree seedlings of *Acer rubrum* L. and *Pinus palustris* Miller, respectively, to evaluate: (1) the availability of soil organic and inorganic N compounds, (2) the uptake of these compounds by plants growing in the field, and (3) the relationship between soil N availability and *in situ* plant N uptake rates. Soil N availability was characterized by determining soluble N concentrations (soluble organic N, ammonium-N, nitrate-N), rates of soil N immobilization, and gross N mineralization rates. Soils that did not contain plants were also used to examine the effect of plant (root) presence on soil N availability. Plant *in situ* uptake of organic and mineral N was assessed by injecting solutions containing stable isotope-labeled ^{15}N-[2]-^{13}C-glycine and ^{15}N-ammonium sulfate into the rhizospheres of seedlings of each species. At approximately one hour and 24 hours following N application, stable isotope compositions of roots and shoots were measured and compared to background tissue isotope values for each species. Measurements of soil N availability were then compared to plant N uptake rates for each species to assess the ability of plant roots to acquire N.

MATERIALS AND METHODS

Study sites

The study was conducted in July 2003 at a bottomland hardwood forest (floodplain) site adjacent to Meyers Branch (a second-order blackwater stream) and at a longleaf pine-scrub oak forest (sandhills) site located on the U.S. Department of Energy's Savannah River Site (SRS; Aiken, SC, USA). The SRS is located on the Upper Atlantic Coastal Plain of south-central South Carolina, and both forest stands typify common ecosystems found in this physiographic province. The climate is humid and temperate with short, mild winters and long, warm summers. The average annual precipitation is approximately 120 cm, and mean daily air temperatures range from 7°C in January to 27°C in July with a mean annual air temperature of 18.2°C (Hunter 1990).

The floodplain soils are sandy, siliceous, thermic Cumulic Humaquepts (Pickney series) and are frequently flooded during the rainy season (Davis and Janecek 1997). These sandy loams are poorly drained, acidic (pH 3.6 to 5.5), and highly organic (Rogers, 1990). The floodplain forest overstory is dominated by *Taxodium distichum, Nyssa biflora,* and *Acer rubrum* tree species. The sandhills soils are thermic, coated Typic Quartzipsamments (Lakeland series), and are very deep, excessively drained, and highly permeable (Davis and Janecek 1997). Sandhills soils are formed from marine, eolian, or fluvial sands, and those at the study site fall under the sand to loamy sand texture class. Sandhills overstory vegetation is dominated by *Pinus palustris*, *Quercus laevis, Q. incana,* and *Q. margaretta.* General soil characteristics (0-15 cm) from both sites are summarized in Table 5.1. Values for soil temperatures, soil pH, total C and total N are from a concurrent two-year soil study conducted at these sites (*unpublished data*).

Nitrogen treatment application and sampling

At each site, thirty seedlings of either *Acer rubrum* (floodplain) or *Pinus palustris* (sandhills) and surrounding soil were carefully removed with a soil corer to 15 cm depth and transplanted intact into PVC sleeves (10 cm dia.). Each intact soil core plus sleeve was then returned to the location where soil had been removed. Seedling sizes per species were similar for all treatments, and biomass values are shown in Table 5.2. After plants were allowed to equilibrate in the sleeves for two weeks, N treatments were randomly assigned to soil cores at each site: 1) background treatment (no water added, no N added; n=6); 2) deionized water treatment (DI; water added, no N added; n=9); 3) ^{15}N-ammonium sulfate treatment (atom%^{15}N = 5%; water added, N added; n=9); 4) ^{15}N-[2]-^{13}C-glycine treatment (atom%^{15}N = 5%, atom%^{13}C = 5%; water added, N added; n=6) (Table 5.3). Of the nine cores that received the ^{15}N-ammonium treatment, six were paired for gross N mineralization determination. Cores in each pair were spatially adjacent to one another (0.5-1 m apart). Pairing was necessary to maintain homogeneity of soil enrichment between cores and reduce possible errors in gross N mineralization rate determination.

Treatment solutions were injected into soil cores using a 15 cm, 18-gauge double-sideport spinal syringe (Popper and Sons, Hyde Park, New York) and an injection template arranged as a uniform x-y grid. Cores plus sleeves were left in the ground, and solutions were injected at twelve points per core. Syringes were simultaneously raised and turned while injecting to ensure uniform labeling and minimal soil disturbance. To prevent contamination of shoots, shoots exposed above the injection template were sealed in a plastic bag during injection, and the bag was carefully removed after treatment application. Injection times were approximately 10-12 minutes per core.

Injection volumes were adjusted for each soil type to limit increases in soil moisture to <5% change in gravimetric soil moisture content (g water g^{-1} dry soil). For each soil type, total solution volume was divided by 12 for injection of identical solution volumes at each of the injection points. Solution concentrations were adjusted per soil type such that application of N to each core added approximately 20 μg N g^{-1} dry soil (~1.1 μg ^{15}N g^{-1}) for the ^{15}N-ammonium treatment and 10 μg N g^{-1} dry soil (~0.6 μg ^{15}N g^{-1}) for the ^{15}N-[2]-^{13}C-glycine treatment. At each site, cores treated with DI water were injected with volumes equivalent to cores injected with N. Background cores receiving no additions were used to provide background plant stable isotope signatures and soil N concentrations. In addition, four cores without plants were randomly assigned a treatment (background, DI, ^{15}N-ammonium sulfate, ^{15}N-[2]-^{13}C-glycine) and injected using the same methods as above (n=16 total). Similar to cores with plants, the four cores without plants in the ^{15}N-ammonium treatment were paired for the determination of gross N mineralization rate. Cores without plants were used to assess whether plant presence in the soil influenced soil N concentrations and mineralization rates.

A subset of soil cores with and without plants was removed within one hour of N application (time = T_1) and sampled to provide initial soil concentrations for gross N mineralization rate determination (Table 5.3). For ^{15}N-ammonium-treated cores with plants, three background cores and one core from each of the three paired cores were sampled per species. In addition, two background cores and one core from each pair of ^{15}N-ammonium-treated cores without plants was sampled at each site. Two soil cores with no plants were also sampled from each DI water and ^{15}N-[2]-^{13}C-glycine treatments to assess short-term effects of watering and organic N immobilization at each site. Twenty-two to 24 hours later (time = T_2),

the remaining cores with and without plants were removed and sampled to evaluate changes in soil N concentrations and stable isotope compositions of plant tissues over one day.

Soil soluble N concentrations

At T_1 and T_2, soil from each core was thoroughly homogenized in a plastic bag in the field, and one scoop of soil was added to a pre-weighed specimen cup (performed in triplicate) containing 70 mL of 2M KCl and placed on ice for transport to the laboratory. Time between N-injection and core sampling was recorded. In the laboratory, the bags of homogenized soils were sub-sampled for determination of gravimetric soil moisture content, and specimen cups were re-weighed to determine the mass of fresh soil added to each cup. Each soil slurry was filtered through Whatman no. 42 filter paper into another sterile specimen cup, and two 15 mL aliquots of each filtered extract solution were removed for determinations of soil N concentrations. The remaining solution volume was recorded and used for the determination of gross N mineralization rate (see below).

For one aliquot, continuous-flow colorimetry was used to determine ammonium concentrations (phenol-hypochlorite assay; U.S. Environmental Protection Agency (EPA) method 353.2) and nitrate concentrations (cadmium reduction assay; EPA method 350.1) at the University of Georgia Institute of Ecology's Soil Isotope/Soil Biology Analysis Laboratory (Athens, Georgia, USA). The precision of ammonium and nitrate measurements was $<\pm 2\%$ for both analytes, and all data were corrected using known reference values in each analytical run. The second aliquot was analyzed using a modified colorimetric ninhydrin method for total ninhydrin-reactive N (free amino acid-N + free amino sugar-N + ammonium-N; Stevenson 1996). The precision of ninhydrin-N analyses were $<\pm 2\%$, and data were corrected to known

reference values in each analytical run. Soil concentration of soluble organic N was calculated as the difference between the total ninhydrin-reactive N and ammonium-N determinations.

Gross N mineralization rate and glycine mineralization rate

Gross N mineralization rate (*m*) was calculated from two paired soil cores without plants and for three paired cores with plants at each site using the stable isotope pool dilution method. The gross mineralization (ammonification) rate of organic matter is determined by adding ^{15}N-ammonium to the soil and measuring the decline in soil ^{15}N composition over time as soil microorganisms convert naturally abundant $^{14+15}$N from organic matter into ammonium. Gross N mineralization rate is calculated following the pool dilution equation from Kirkham and Bartholomew (1954; notation from Smith *et al.* 1994):

$$m = [(AT_1 - AT_2) / \Delta t] * [\log ((AL_1AT_2) / (AL_2AT_1)) / \log (AT_1/AT_2)],$$

where *m* = mass of N mineralized per unit time per unit mass of soil ($\mu g\ g\ soil^{-1}\ d^{-1}$), AT = $^{14+15}$N-ammonium ($\mu g\ g^{-1}$) and AL = ^{15}N-ammonium ($\mu g\ g^{-1}$) at differing times (T_1, T_2), and Δt = change in time. Values for AT were determined colorimetrically from soils extracted with 2M KCl immediately after ^{15}N-ammonium sulfate application (T_1) and after 22-24 hours (T_2) (previously described).

Ammonium was recovered from T_1 and T_2 filtered 2M KCl extracts for gross N mineralization rate determination using the ammonia diffusion technique described by Brooks *et al.* (1989). Approximately 40 mL of filtered 2M KCl extracts was made alkaline by adding 0.25 g of MgO to each specimen cup to convert dissolved ammonium to ammonia vapor. The vapor

was trapped on a 7 mm disk of Whatman no. 3 filter paper suspended above the solution with stainless steel wire. The ammonia traps were pre-leached with deionized water and acidified with 10 μL of 2.5M $KHSO_4$ (~350 μg N trapping capacity). After MgO was added, each cup was immediately sealed with a tightly fitting screw cap. The solution was then carefully swirled to disperse the MgO, and the cup was set aside at room temperature. After six days, the disks were removed and placed in a dessicator over concentrated H_2SO_4 to dry overnight.

Colorimetric analyses of ammonium remaining in diffused extracts verified that >90% (92±1%) of the ammonium diffused out of solution and that, on average, only 17±1% of the disk trapping capacity was used. After drying overnight, whole disks were loaded into tin cups for $\delta^{15}N$ analysis using a continuous flow isotope mass spectrometer (Delta^{Plus} XL, Thermo-Finnegan) coupled to an elemental analyzer (CN2500, CE Instruments). Of the 92±1% of the ammonium that diffused from extracts, 97±2% was recovered in the acid traps. For all samples analyzed using isotope mass spectrometry, measurement precision was ±0.07‰ ($\delta^{13}C$) and ±0.05‰ ($\delta^{15}N$), and ±0.4% and ±0.1% for percent C and N, respectively. All isotope and percent measurements were corrected using known reference values in each analytical run.

To assess the mineralization of ^{15}N-[2]-^{13}C-glycine label to ^{15}N-ammonium at T_1 and T_2, extracts of glycine-treated cores were also diffused. The amount of glycine mineralized was calculated from the atom percent excess of ^{15}N-ammonium in glycine-treated soils compared to background soils. Glycine mineralization rates for each site at T_1 and T_2 were calculated as μmol ^{15}N-ammonium g^{-1} day $(d)^{-1}$ for soils. Molar mineralization rates were then converted to mass values (μg ^{15}N-ammonium g^{-1} d^{-1}) for comparison with gross N mineralization rates and other soil N measurements.

Plant tissue isotope composition and in situ N uptake

For T_1 and T_2 cores with plants, shoots were carefully removed to prevent contamination with the isotope label applied to soils. Coarse roots and attached fine roots were removed from the soil by hand and rinsed three times with 0.5 mM $CaCl_2$ to remove excess label from root surfaces. Detached coarse and fine roots in the bulk soil were discarded because their origin could not be positively identified. Roots and shoots were stored separately in plastic bags, placed on ice for transport to the laboratory, and dried at 50°C for 72 hours. Dried plant material was ground to a fine powder at –200°C and loaded into tin cups for $\delta^{13}C$ and $\delta^{15}N$ analyses. Duplicate root and shoot samples were analyzed for each plant used in the study.

Changes in ^{15}N and ^{13}C concentrations (excess μmol ^{15}N or ^{13}C g^{-1}) in roots and shoots at T_1 (ammonium-treatment only) and T_2 (all N treatments) were calculated for each species per treatment as the difference between the atom percents of each isotope in background and treated tissues. Excess molar isotope concentrations were then converted to mass values (excess μg ^{15}N or ^{13}C g^{-1}) for comparison with other soil N measurements. Root uptake rates of ^{15}N and ^{13}C for plants in DI water, ammonium, and glycine treatments were calculated per unit dry mass per day (excess μg ^{15}N or ^{13}C g^{-1} d^{-1}).

Statistical analyses

To avoid violating assumptions of normality and homoscedasticity, all statistical tests used in this study were nonparametric analyses of variance. Pair-wise differences between sites, treatments, times, and species were determined using Mann-Whitney *U* tests. Differences between multiple groups were analyzed using Kruskal-Wallis tests, followed by nonparametric Tukey-type multiple comparisons. For groups of equal sample size, the nonparametric Nemenyi

multiple comparison test was used, and Dunn's nonparametric multiple comparison test was used for groups of different sample sizes (Zar 1996). All statistical tests were considered significant at the α=0.1 level.

Soil N measurements were pooled by time and then by site for background and DI-treated cores with and without plants because there were no significant treatment effects (total background *n*=23 per site). Similarly, tissue ^{15}N and ^{13}C contents did not differ significantly between background and DI-treated plants or between T_1 and T_2 plants for each species, so they were pooled by species as well (hereafter referred to as "background"; *n*=15). Because ^{15}N-ammonium and ^{15}N, [2]-^{13}C-glycine treatments were added at different N concentrations, changes in soil N or tissue isotope compositions in each treatment were compared to background values only for each site or species.

To evaluate potential relationships between background soil N concentrations and other soil variables, N concentrations were regressed against other measured soil properties (*n*=23 per site). Regressions of soil N concentrations and soil moisture content, however, were constructed for untreated background cores only (n=10) since water was added to soils in DI-treated cores. All concentrations are reported per unit dry mass soil or plant tissue, and values are reported as treatment means ± one standard error (s.e.) unless otherwise noted.

RESULTS

Background soil N concentrations

In both floodplain and sandhills soils, the average background soil concentration ($\mu g\ N\ g^{-1}$) of soluble N was greatest for SON, followed by ammonium-N, and then nitrate-N (Fig. 5.1). Soluble organic N concentrations were significantly higher than ammonium-N in sandhills soils

(3.6±0.3 μg SON g^{-1} and 1.3±0.2 μg NH_4^+-N g^{-1}, respectively) but were not different in floodplain soils (11.6±1.1 μg SON g^{-1} and 9.0±1.2 μg NH_4^+-N g^{-1}, respectively). Both SON and ammonium-N concentrations were significantly greater in floodplain compared to sandhills soils. Nitrate N concentrations were significantly lower than SON and ammonium-N at each site (floodplain, 0.52±0.27 μg NO_3^--N g^{-1}; and sandhills, 0.10±0.01 μg NO_3^--N g^{-1}), but they did not differ between sites.

In untreated background soils (*n*=10), soil moisture (% mass) ranged from 51.4-76.0% (64.8±1.5) and 17.4-25.6% (19.7±0.4%) in floodplain and sandhills soils, respectively. Soluble organic N and ammonium-N concentrations showed a significant positive correlation to soil moisture content in floodplain soils only (Fig. 5.2). For all background soils (*n*=23 per site), SON and ammonium-N showed significant positive correlations in both floodplain and sandhills soils (Table 5.4). Significant positive correlations were also observed for both SON and ammonium-N concentrations when each was regressed against nitrate-N in sandhills soils. For floodplain soils, there was a significant negative correlation between ammonium-N and nitrate-N concentrations while no significant relationship was apparent between SON and nitrate concentrations.

Gross N mineralization, glycine mineralization, and soil N immobilization

Gross N mineralization rates determined from ammonium-N treated soils were significantly higher in sandhills soils (m_S=3.7±0.3 μg N g^{-1} d^{-1}; *n*=5) than floodplain soils (m_F=0.7±1.8 μg N g^{-1} d^{-1}; *n*=5). Mineralization rates (mean±standard deviation) of ^{15}N-[2]-^{13}C-glycine were 0.47±0.44 μg ^{15}N g^{-1} d^{-1} and 0.69±0.60 μg ^{15}N g^{-1} d^{-1} in floodplain and sandhills T_1 soils (*n*=2 per site; no plants), respectively (Fig. 5.3). In T_2 floodplain and sandhills soil cores

without plants, glycine mineralization rates were 0.07±0.02 μg ^{15}N g^{-1} d^{-1} and 0.010±0.004 μg ^{15}N g^{-1} d^{-1}, respectively (n=2 per site), and were not significantly different from cores with plants (floodplain: 0.06±0.01 μg ^{15}N g^{-1} d^{-1}; sandhills: 0.03±0.01 μg ^{15}N $g^{-1}d^{-1}$; n=6 per site). Soil cores with plants at T_2, however, were different between sites. Statistical comparisons using values from T_1 and T_2 cores without plants were not possible due to small sample size. For comparison with gross N mineralization rates, T_2 glycine mineralization rates were pooled for T2 cores with and without plants (n=8 per site) since plant presence had no statistically significant treatment effect on rates.

The increases in floodplain and sandhills soil concentrations after ^{15}N-ammonium treatment (treated – background) at T_1 approximated the N application at both sites within the variability of the measurements (Fig. 5.4). Floodplain soil concentrations of ammonium-N were not significantly different between T_1 and T_2, but sandhills soils immobilized a significant amount of added label between T_1 and T_2 (~68%). The rates of ammonium-N immobilization were significantly higher at T_1 than T_2 in both soils, and significantly higher in floodplain than sandhills soils at T_1 only. In ^{15}N-[2]- ^{13}C-glycine treatments, similar calculations of glycine immobilization rates could not be performed because SON determinations were not glycine-specific.

Applications of ammonium and glycine affected soil concentrations of all soluble N compounds relative to background concentrations at one or both sites (Fig. 5.5). Furthermore, changes in soil concentrations relative to background varied between cores with and without plants at both sites. Ammonium applications increased T_1 soil ammonium-N concentrations significantly in cores with and without plants at both sites, and all cores remained significantly higher than background values at T_2. Ammonium applications to floodplain soils had no effect

on SON concentrations in all cores, but significantly increased SON concentrations in all sandhills T_1 cores and in T_2 cores with plants only. Nitrate-N concentrations were significantly higher in ammonium-treated floodplain T2 soils with plants, but were not different than background concentrations in sandhills soils. Glycine applications significantly increased SON concentrations relative to background at T_1 and had no effect on T_1 ammonium-N or nitrate-N concentrations at either site. Soluble organic N concentrations remained significantly higher than background in all T2 soils except floodplain soils with plants. Glycine-treated floodplain soils at T2 increased ammonium-N concentrations in cores without plants and nitrate-N concentrations in cores with plants. At T2, ammonium-N concentrations in glycine-treated sandhills soils with plants were significantly higher than background.

Plant isotope compositions and in situ N uptake rates

Root ^{15}N concentrations in both *A. rubrum* and *P. palustris* were significantly in excess of background values for T_1 and T_2 plants treated with ^{15}N-ammonium sulfate (Fig. 5.6). Excess root ^{15}N appeared higher at T_2 than T_1 in both species but was only significant for *P. palustris,* and concentrations were significantly different between species for T_1 only. *Acer rubrum* shoot ^{15}N concentrations in ammonium-treated T_1 and T_2 plants were also significantly in excess of background, however, there was no significant difference between excess shoot ^{15}N for T_1 and T_2 plants. *Pinus palustris* shoots showed significant excess ^{15}N shoot concentrations in T_2 plants only. In both *A. rubrum* and *P. palustris* plants treated with ^{15}N-[2]-^{13}C-glycine, root ^{15}N concentrations were significantly in excess of background. Excess shoot ^{15}N concentrations, however, were significant only for *A. rubrum*.

Excess ^{13}C tissue concentrations of glycine-treated plants appeared greater than background values of both species, but were significantly higher for *A. rubrum* and *P. palustris* shoots only. Although no ^{13}C was applied in the ^{15}N-ammonium sulfate treatment, root and shoot ^{13}C concentrations were significantly in excess of background values for *P. palustris* shoots at T_2 only. In addition, for plants treated with ammonium, excess ^{13}C concentrations in *A. rubrum* shoots were significantly higher than in *P. palustris* shoots at T_1.

Root *in situ* ^{15}N uptake rates for *A. rubrum* and *P. palustris* were significantly higher at T_1 (5.6±1.2 and 5.7±0.5 μg ^{15}N g^{-1} d^{-1}, respectively) than T_2 (0.5±0.1 and 1.0±0.2 μg ^{15}N g^{-1} d^{-1}, respectively) in ammonium-treated plants (Table 5.4). Uptake rates of ^{15}N were not significantly different between species per N treatment. Root uptake rates of ^{15}N in glycine-treated soils approximated those in ammonium-treated soils, but treatments were not statistically comparable for each species or between species because N application rates were different.

DISCUSSION

Background soil N availability

Soil amino acids constitute part of the soil soluble organic N (SON) pool that is easily mineralizable (Murphy *et al.* 2000), and so play important roles in soil N transformations and, subsequently, plant nutrition (Mengel *et al.* 1999). In this study, the ratio of average SON, ammonium-N, and nitrate-N concentrations in floodplain soils was approximately 22:17:1. The predominance of SON and ammonium-N in floodplain soils is consistent with studies reporting similar patterns in soil N concentrations for acidic organic soils in cooler alpine and arctic ecosystems (Kielland 1995, Nordin *et al* 2001, Jones and Kielland 2002) and warmer subtropical ecosystems (Schmidt and Stewart 1997) where low temperatures and/or low pH limit

mineralization processes and lead to the build-up of organic matter. High water tables and periodic flooding in temperate floodplains cause soils to be reduced, often decreasing soil pH and increasing organic matter accumulation by limiting microbial mineralization processes (Mitsch and Gosselink 2000, p. 536; Updegraff *et al.* 1995).

For the floodplain soils used in this study, the significant positive relationship between SON concentration and soil moisture content supports this idea (Fig. 5.2), but a significant positive correlation was also observed for ammonium-N concentrations. Higher SON concentrations with increasing soil moisture likely provided more organic substrate for mineralization, resulting in the observed positive correlations between ammonium-N and both SON (Table 5.4) and soil moisture content. The negative relationship between soil moisture and nitrate-N was not significant, but greater potential losses of nitrate via denitrification in more saturated soils may have driven the negative correlation between ammonium-N and nitrate-N. Although we did not explicitly test the effect of soil moisture level on mineralization or nitrification/denitrification rates, our data suggest that soil moisture levels in temperate floodplain ecosystems strongly impact both soluble organic and inorganic N dynamics.

Soluble N concentrations in sandhills soils were generally 3-7 times lower than in floodplain soils (Fig. 5.1). Interestingly, the ratio of average SON, ammonium-N, and nitrate-N concentrations in sandhills soils was approximately 38:13:1, similar to the distribution pattern of soluble N compounds in floodplain soils. In contrast to floodplain soils, SON concentrations were significantly higher than ammonium-N concentrations in sandhills soils, in part because these coarse-textured, low organic matter soils do not bind ammonium and inorganic N is easily leached (Appel and Mengel 1993). In addition, all pair-wise regressions of SON, ammonium-N, and nitrate-N concentrations were positively correlated, suggesting that mineralization and

nitrification rates are substrate-limited (organic N and ammonium-N, respectively), and that rates increase with increasing substrate availability in sandhills soils (Table 5.3).

Soil N mineralization

The higher rates of gross N mineralization in sandhills soils (m_S: 3.7±0.3 μg N g^{-1} d^{-1}) compared to floodplain soils (m_F=0.7±1.8 μg N g^{-1} d^{-1}) support the interpretation that saturated soil conditions limit N transformation rates (Updegraff *et al.* 1995, Schmidt and Stewart 1997, Wang *et al.* 2001). In addition, high variability in m_F also reflects the highly variable ammonium-N concentrations measured in replicate floodplain cores. As noted earlier, N concentrations in these floodplain soils are strongly influenced by soil moisture, and soil moisture varied widely among replicate cores in this study (51-76%). The large range in soil moisture was likely due to slight differences in ground elevations that affected infiltration path lengths and/or varied soil depth to the water table. Since water table levels themselves also vary, small changes in ground elevation may translate into significant differences in the magnitude and duration of soil saturation between adjacent soil microsites (Mitsch and Gosselink 2000, p. 536).

Short-term (T_1) mineralization rates of ^{15}N -[2]-^{13}C-glycine to ^{15}N-ammonium in floodplain and sandhills soils appeared higher than glycine mineralization rates in floodplain and sandhills soils at T_2 (Fig. 5.3). Although these observations were not statistically comparable because n=2 for both T_1 and T_2 soils without plants, the large standard deviations of each average suggest that soil variability of glycine mineralization rates is very high. The high rates are also consistent with recent studies reporting very rapid turnover times for soil amino acids, ranging from 1 to 28 hours (Lipson *et al.* 2001, Jones and Kielland 2002, McFarland *et al.* 2002, Henry and Jeffries 2003b).

To allow comparison to gross N mineralization rates (pooled by site for cores with and without plants), values for T_2 were also pooled by site (n=8) since rates did not differ between cores with and without plants. Rates of glycine mineralization in T_2 soils (floodplain: 0.06±0.01 μg ^{15}N g^{-1} d^{-1}; sandhills: 0.03±0.01 μg ^{15}N g^{-1} d^{-1}) were significantly lower than in T_1 soils at both sites although substantially more glycine was mineralized in T_2 soils (floodplain: 10.4±1.4%; sandhills: 4.2±1.3%) compared to T1 (~1% in both sites). The significant site differences in the proportion of label mineralized at T_2 led to significantly lower mineralization rates in sandhills compared to floodplain soils, contrasting with gross N mineralization results that show the opposite relationship ($m_F < m_S$).

The inconsistencies of glycine mineralization rate patterns compared to gross mineralization rate patterns are likely due to the character of the organic nitrogen pool at each site (see Table 5.1). Gross mineralization rates were determined from the dilution of added product (^{15}N-ammonium) over time, whereas glycine mineralization was determined from the transformation of added substrate (^{15}N-[2]-^{13}C-glycine) to ammonium over time. The composition and mineralization of individual organic compounds may differ greatly between sites, but because the sizes of mineralizable organic N pools differed between sites, other mineralization effects may have obscured those quantified for glycine in this study.

Although plants had no effect on either gross N mineralization rates or glycine mineralization rates in both soils, the presence of plant roots significantly affected T_2 soil soluble N in one or both sites relative to background concentrations (Fig. 5.5). In floodplain soils, ammonium additions had no effect on nitrate-N concentrations in soils with or without plants, indicating that nitrification in floodplain soils was probably not limited by substrate (ammonium) availability. Nitrate-N concentrations, however, increased after the addition of glycine to soils

with plants only, suggesting that nitrification (an aerobic transformation) was likely C-limited and occurred only when anoxic soils conditions were ameliorated by the presence of roots. In sandhills soils, significant increases in SON and ammonium concentrations in response to ammonium and glycine treatments, respectively, occurred only in cores with plants, suggesting that plant roots provided more favorable environments for microbial transformations compared to the bulk soil.

Soil N immobilization

Overall, short-term (T_1) immobilization of added $^{14+15}$N-ammonium (^{15}N-ammonium) was not different between sites, but a small fraction of label appeared to be immobilized by T_1 floodplain soils (~15%) (Fig. 5.4). Patterns of N immobilization were likely affected by the increase in soil ammonium-N concentrations relative to background concentrations following N application at each site. Although equivalent rates of ammonium-N were injected into both floodplain and sandhills soils, N applications tripled the background ammonium-N concentrations in floodplain soils but increased sandhills background concentrations by approximately 19 times.

Significantly lower background ammonium-N concentrations suggest that sandhills soils are more N-limited than floodplain soils, but sandhills soils did not retain a significant amount of added $^{14+15}$N-ammonium (^{15}N-ammonium) within one hour of N application presumably due to significantly less adsorption than floodplain soils. After 24 hours (T_2), however, sandhills soils immobilized ~68% of the added label while floodplain soils showed no additional immobilization. The significant increase in immobilzation at T_2 coupled with the lack of immobilization at T_1 in sandhills soils suggests that more N was available for a longer period of

time, resulting in the significant immobilization of added N. Most of the ammonium-N retained by sandhills soils at T_2 was probably sequestered by microorganisms since significant physical adsorption seems unlikely in these coarse-textured, low organic matter soils (Appel and Mengel 1993). As a result of increased N availability, microbial activity was likely stimulated, contributing to the higher observed rates of gross N transformations in sandhills soils than floodplain soils. In floodplain soils, the lower relative increase in N concentration coupled with the significantly higher short-term immobilization rate and lack of additional immobilization over time suggests that: 1) the small fraction of added N retained was mostly due to physical adsorption of ammonium to the soil instead of biological immobilization, and 2) soil microbes were not N-limited, or microbial N uptake was limited by some other nutrient.

Plant in situ uptake and soil competition for soluble inorganic N

Changes in tissue isotope compositions after soils were injected with isotopically labeled ammonium and glycine varied for *A. rubrum* and *P. palustris* and were related to site-specific soil characteristics. In the ^{15}N-ammonium sulfate treatment, root ^{15}N concentrations from both species were significantly in excess of species background values at both T_1 and T_2 (Fig. 5.6). The short-term (T_1) increase in *A. rubrum* root ^{15}N concentration was significantly higher compared to *P. palustris* roots, but root ^{15}N uptake rates did not differ between species (Table 5.4). Since the average root biomass of *A. rubrum* plants was much lower than that of *P. palustris* (Table 5.2), equivalent rates of ^{15}N uptake in both species resulted in significantly higher ^{15}N concentrations in *A. rubrum* roots. Furthermore, greater potential mobility of added ^{15}N-ammonium in waterlogged floodplain soils coupled with better distributed, more highly branched root structure in *A. rubrum* (compared to *P. palustris* taproots) also likely contributed

to the significantly higher ^{15}N concentrations in *A. rubrum* roots. Significant excess ^{15}N concentrations in *A. rubrum* T_1 shoots indicate that the ^{15}N-ammonium taken up by roots was rapidly translocated aboveground. Given that floodplain soils adsorbed most of the added ammonium within one hour (Fig. 5.4), the significant increase in root excess ^{15}N concentrations during T_1 suggests that *A. rubrum* roots compete well against soil immobilization factors during flushes of increased ammonium. Once ammonium was immobilized in floodplain soils, however, uptake rates of ^{15}N by *A. rubrum* roots dropped significantly and no further increases in root or shoot ^{15}N concentrations were observed at T_2.

In contrast, excess ^{15}N concentrations in *P. palustris* roots were significantly higher at T_2 relative to T_1 (Fig. 5.6), despite the large amount of added ammonium immobilized by sandhills soils (Fig. 5.4). Similar to *A. rubrum*, root ^{15}N uptake rates in *P. palustris* at T_1 were high in response to the flush of ammonium and decreased significantly by T_2 (Table 5.4). Soil moisture content of sandhills soils was relatively low, suggesting that mobility of added ammonium was limited and potentially localized plant-microbial competition for N to areas directly injected with treatment solution. Sandhills soils lacked significant quantities of organic matter or clay that could adsorb added N and the increase in ammonium-N was high relative to background soil concentrations (19 times greater); thus, *P. palustris* roots likely had greater success acquiring added ^{15}N-ammonium over time, resulting in the significantly higher excess ^{15}N concentrations at T_2 compared to T_1. The ^{15}N-ammonium taken up by roots at T_1 was not apparent in T_1 shoots, but this could be due to significantly higher average shoot biomass of *P. palustris* compared to *A. rubrum* (Table 5.2). By T_2, shoot ^{15}N concentrations were significantly in excess of background shoot values.

Patterns of *in situ* ^{15}N-ammonium uptake rates and excess ^{15}N concentrations in *A. rubrum* and *P. palustris* roots were variable compared to different measurements of soil N availability. Both ammonium concentrations and glycine mineralization rates were significantly higher in floodplain soils compared to sandhills soils (Figs. 5.1, 5.3), suggesting that greater availability would be reflected in higher uptake rates in *A. rubrum* compared to *P. palustris* roots. In contrast, rates of gross N mineralization ($m_F<m_S$) predicted the opposite pattern in plant uptake rates (*A. rubrum*<*P. palustris*). Interestingly, uptake rates of ^{15}N-ammonium did not differ between species at either time, and patterns in root excess ^{15}N concentrations were more strongly influenced by other soil properties that controlled N availability (texture, adsorption, soil moisture). Overall, our results are consistent with other studies showing that patterns in plant N use cannot be predicted by indices of N availability alone (Raab *et al.* 1996, Persson and Näsholm 2002, Miller and Bowman 2003).

Plant in situ uptake and soil competition for soluble organic N

Twenty-four hours following soil application of ^{15}N-[2]-^{13}C-glycine, concentrations of ^{15}N were significantly in excess of background in both *A. rubrum* and *P. palustris* roots, but only *A. rubrum* shoots exhibited significant concentrations of excess ^{15}N (Fig. 5.6). Root uptake rates of ^{15}N-glycine were not significantly different between species. Although glycine-treated soils received only half of the N applied in ammonium-treated soils, ^{15}N uptake rates in glycine-treated plants at T_2 approximated ^{15}N uptake rates in ammonium-treated plants of both species (Table 5.4).

As noted earlier, both soils mineralized added glycine, and ammonium concentrations in glycine-treated T_2 soils were significantly higher than background concentrations in both

floodplain and sandhills soils. It therefore seems likely that the increases in plant tissue ^{15}N concentrations from soils treated with ^{15}N-[2]-^{13}C-glycine were derived mainly from mineralized glycine label instead of the uptake of intact glycine molecules. The absence of significant intact glycine uptake in *A. rubrum* and *P. palustris* is further evidenced by a lack of significant excess ^{13}C concentrations in roots of both species (Fig. 5.6). Because only one carbon atom (C2 position, *i.e*. amino end) was isotopically labeled for every N atom in the glycine label, we would expect a 1:1 molar enrichment of ^{13}C:^{15}N in roots if uptake of intact glycine had occurred. We would not expect the same molar enrichment ratio in shoots because the translocation of N from roots to shoots occurs in the form of specific amino acids (amides, asparagines, glutamine) (Näsholm and Persson 2001, Henry and Jefferies 2003b).

Shoot ^{13}C concentrations, however, were significantly in excess of background values for both *A. rubrum* and *P. palustris* in glycine-treated plants. Interestingly, T_2 shoots of ammonium-treated *P. palustris* plants also showed significant excess ^{13}C concentrations although no ^{13}C was experimentally added. The potential exists that the observed increases in *A. rubrum* and *P. palustris* tissue excess ^{13}C contents were likely indirect treatment effects rather than the result of direct physiological responses to treatment ^{13}C additions.

No significant increases in tissue ^{13}C concentration occurred in plants of either species treated with DI water only, suggesting that the ^{13}C enrichment response could not be due to watering. Specifically, the change in soil moisture from treatment solutions was not sufficient to alter stomatal openness that would have increased the rate of CO_2 diffusion into shoots, thereby increasing tissue ^{13}C. Plant productivity was likely N-limited, so N additions may have stimulated photosynthetic rates in both species, shifting the photosynthetic rate-limiting factor from N to C limitations via CO_2 diffusion rate. As a result, discrimination against ^{13}C during

carbon fixation was reduced in *A. rubrum* and *P. palustris* shoots and tissue ^{13}C concentrations increased. Alternatively, some of the excess ^{13}C detected in shoots of glycine-treated plants may have been derived from photosynthetic fixation of $^{13}CO_2$ respired from the soil during microbial transformations of added ^{15}N-[2]-^{13}C-glycine. We could not confirm this, however, since evolution of $^{13}CO_2$ from soils was not measured. Regardless, translocation of ^{13}C-enriched photosynthate belowground likely caused the slight, but not statistically significant, increases in excess root ^{13}C concentrations in both *A. rubrum* and *P. palustris.*

CONCLUSIONS

Soil concentrations of SON equaled or exceeded inorganic N concentrations in soils from temperate bottomland hardwood forest (floodplain) and longleaf pine/scrub oak forest (sandhills). Roots of both *Acer rubrum* L. and *Pinus palustris* Miller demonstrated the ability to compete well against soil N immobilization factors for available ammonium-N in floodplain and sandhills soils, respectively, while they did not use N or C applied as the amino acid glycine. Relationships between N concentrations and N mineralization rates were strongly influenced by soil moisture in floodplain soils and differed from patterns of N availability in sandhills soils. In addition, the relationships between soil N availability and plant N uptake were highly variable and differed over time, reflecting the complexity of plant N use patterns. Additions of glycine to both floodplain and sandhills soils influenced plant N uptake and possibly plant productivity by providing easily mineralizable N and C. While organic N uptake as glycine was not unequivocally traced in either tree species examined here, soil soluble organic N clearly plays a pivotal role in soil N dynamics and plant N nutrition in these two contrasting temperate ecosystems.

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Figure 5.1. Soil background concentrations of soluble organic N (SON), ammonium-N, and nitrate-N in floodplain and sandhills soils (n=23 per site). Bars and lines represent means and standard errors, respectively. Different lower case letters represent significant differences between soluble N concentrations at each site, and asterisks indicate significant site differences in soil N concentration.

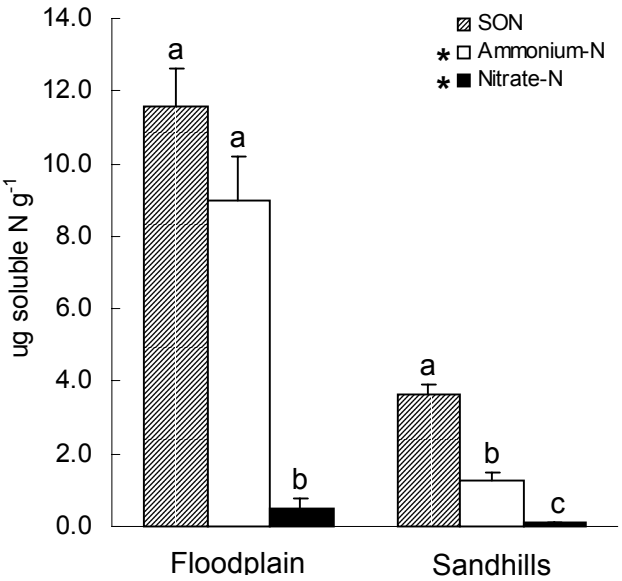
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
ug soluble N g-1
SON
Ammonium-N
Nitrate-N
a
a
b
a
b
c
Floodplain
Sandhills

Figure 5.2. Relationships between soil SON and ammonium-N concentrations and soil moisture content in untreated background floodplain soils (n=10; slope = 0.77, R^2 = 0.96, $P < 0.001$).

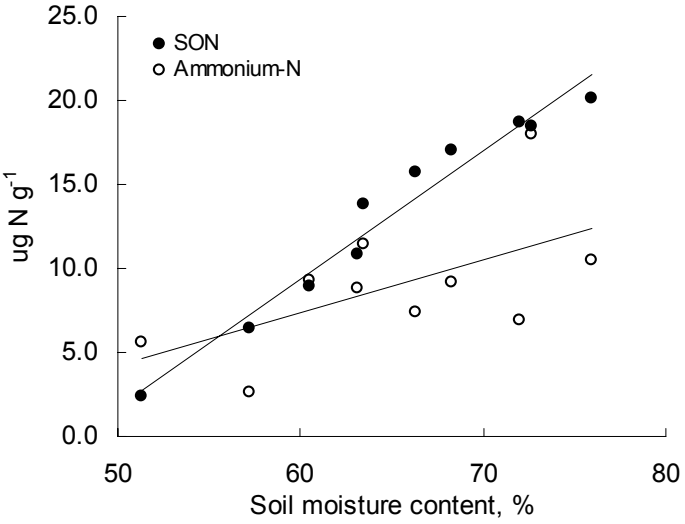
25.0
20.0
15.0
10.0
5.0
0.0
ug N g-1
SON
Ammonium-N
50
60
70
80
Soil moisture content, %

Figure 5.3. Glycine mineralization rates for floodplain and sandhills soils at T_1 (without plants (-), n=2 per site; with plants (+), n=2 per site) and T_2 (with plants; n=6 per site). Bars represent treatment averages at each site. Lines represent standard deviations for cores without plants and standard errors for cores with plants. Different lower (upper) case letters represent significant treatment differences at each site, and the asterisk denotes significant differences between sites.

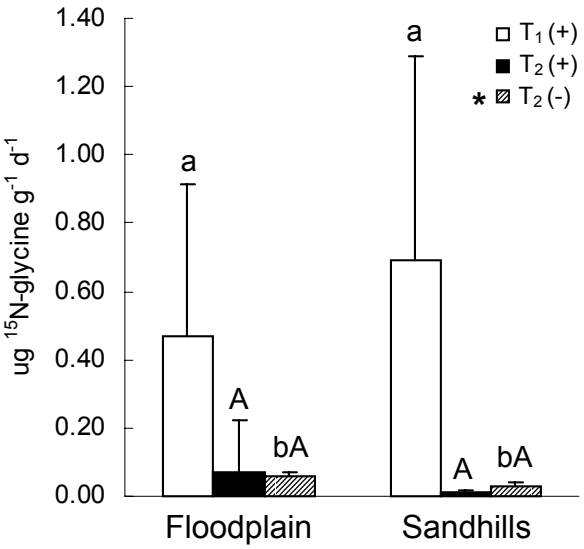
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
ug ^{15}N-glycine g^{-1} d^{-1}
a
A
bA
a
A
bA
T$_1$ (+)
T$_2$ (+)
* T$_2$ (-)
Floodplain
Sandhills

Figure 5.4. Immobilization of added ammonium-N (treatment – background concentrations) at T_1 (*n*=5 per site) and T_2 (*n*=8 per site) in floodplain and sandhills soils. Right panels show amount of ammonium-N immobilized, and left panels show rates of N immobilization. Bars and lines represent site averages and one standard error, respectively, for each time. Dotted lines in the left panels show N application rates. Common lower case letters indicate lack of differences between T_1 and T_2 at each site, and asterisks denote significant differences between sites at each time.

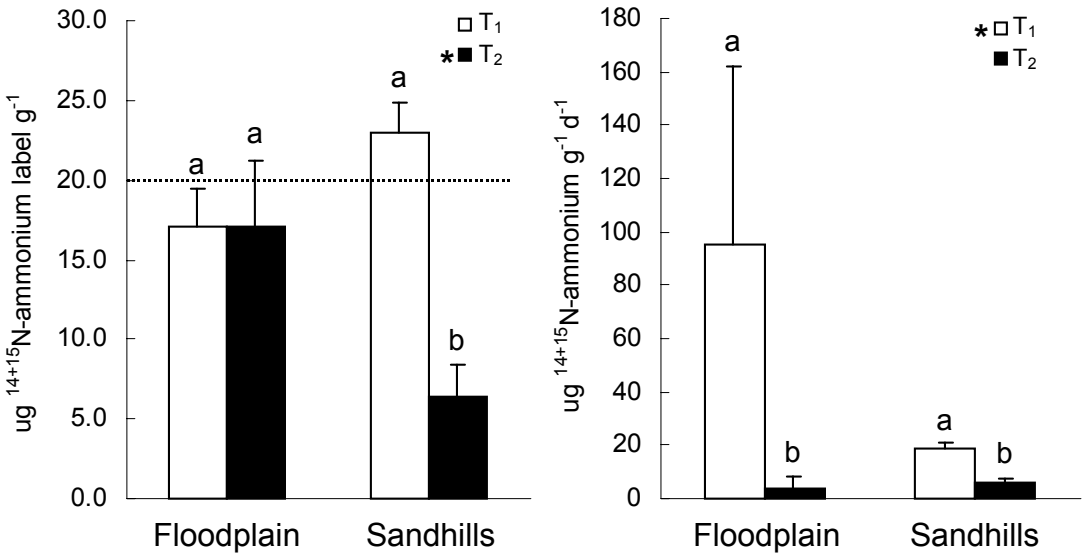
30.0
25.0
20.0
15.0
10.0
5.0
0.0
ug 14+15N-ammonium label g-1
□ T1
* ■ T2
a
a
a
b
Floodplain
Sandhills
180
160
140
120
100
80
60
40
20
0
ug 14+15N-ammonium g-1 d-1
* □ T1
■ T2
a
b
a
b
Floodplain
Sandhills

Figure 5.5. Soil concentrations of SON, ammonium-N, and nitrate-N in floodplain and sandhills soils treated with ammonium (left panels) and glycine (right panels) at T_1 and T_2 without (-) and with (+) plants. Refer to Table 5.3 for *n* per treatment (*nd* no data). Bars represent treatment averages at each site. Lines represent standard deviations when $n=2$ and standard errors when $n \geq 3$. Crosses indicate significant treatment differences from background values.

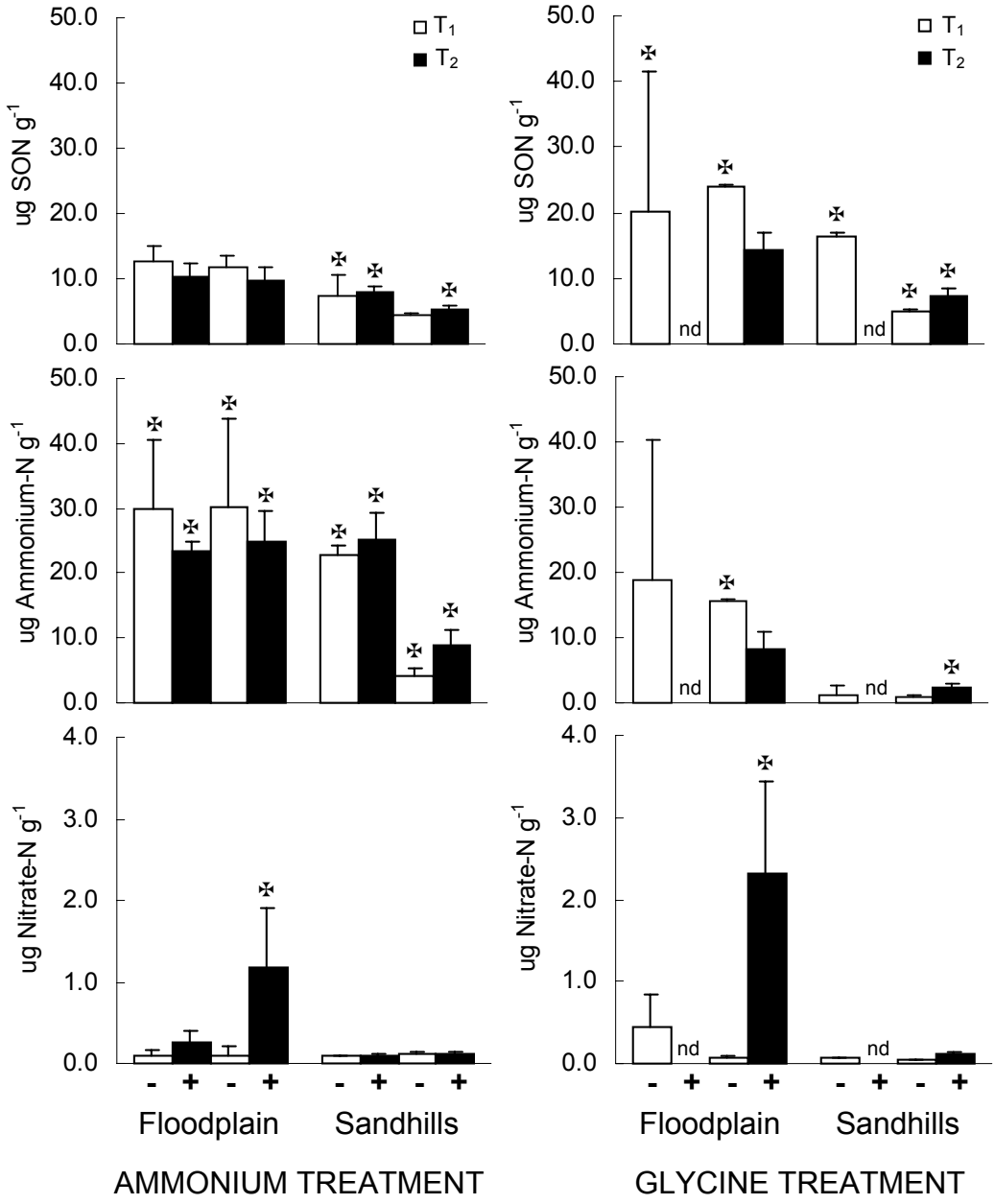
ug SON g-1
ug Ammonium-N g-1
ug Nitrate-N g-1
T1
T2
nd
- + - + - + - +
Floodplain
Sandhills
AMMONIUM TREATMENT
GLYCINE TREATMENT

Figure 5.6. Tissue concentrations of ^{15}N and ^{13}C in excess of background tissue values in *A. rubrum* and *P. palustris* after soil injections of ^{15}N-ammonium sulfate and ^{15}N-[2]-^{13}C-glycine. Bars and lines represent tissue means and one standard error, respectively, per treatment. Crosses indicate significant treatment differences from background values, and asterisks denote significant site differences per treatment. *Bkd*: background (n=15), *T_1 NH_4^+*: T_1 ammonium (n=3), *T_2 NH_4^+*: T_2 ammonium (n=6), *T_2 Gly*: T_2 glycine (n=3). Only *P. palustris* root ^{15}N concentrations were significantly different between T_1 and T_2 ammonium treatments†.

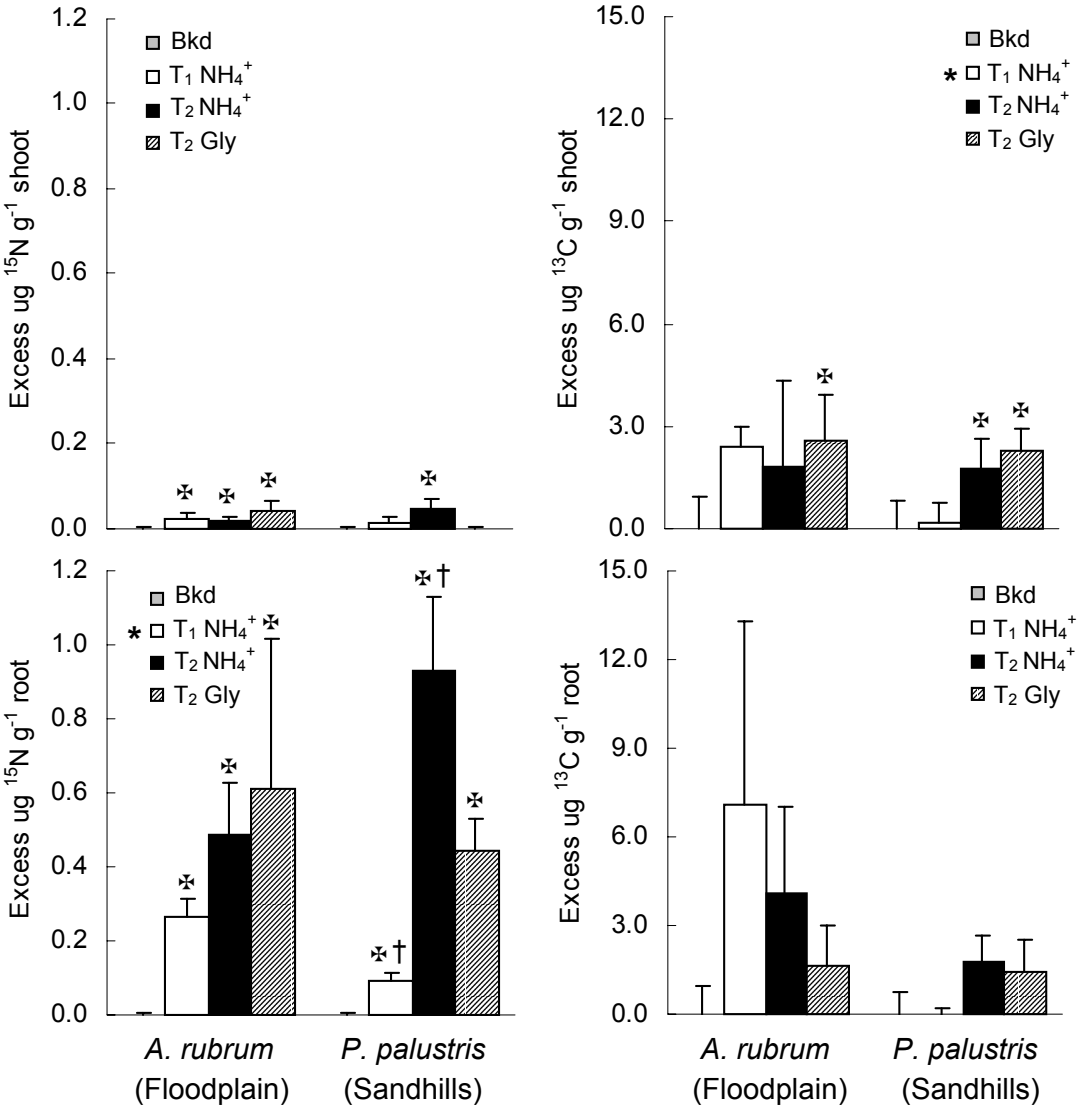
Excess ug 15N g-1 shoot
Excess ug 13C g-1 shoot
Excess ug 15N g-1 root
Excess ug 13C g-1 root
Bkd
T1 NH4+
T2 NH4+
T2 Gly
A. rubrum
(Floodplain)
P. palustris
(Sandhills)

Table 5.1. Summary of soil characteristics from bottomland hardwood floodplain forest (F) and sandhills forest (S) study sites (Aiken, South Carolina). Average soil moisture (one s.e.) was calculated from soil cores used in the present study (*n*=10 per site). Range (average) for soil temperatures taken from July data collected during a concurrent two-year soil study conducted at these sites (*unpublished data*). The same study provided total ranges (averages) for other variables observed over the two-years. Soil pH was measured using a fresh soil to water ratio of 1:3.

Site	Soil moisture	July soil T	pH	Total N	Total C
	---% mass---	--°C at 5 cm--	----1:3----	----------mg g^{-1}----------	
F	64.8 (1.5)	22.5-25.0 (23.7)	3.5-5.2 (4.3)	6.44-11.05 (8.25)	115.5-197.6 (153.8)
S	19.7 (0.4)	23.7-26.9 (25.6)	4.6-5.6 (5.0)	0.35-0.59 (0.42)	8.0-16.4 (11.7)

Table 5.2. Average background values for plant tissue biomasses (± one standard error, *n*=30) and tissue percent carbon, nitrogen, and stable isotope compositions ($\delta^{15}N$, $\delta^{13}C$; *n*=15) from bottomland hardwood floodplain forest (F) and sandhills (S) study sites (Aiken, South Carolina). Because there were no significant differences between background and DI water-treated plants, tissue percent carbon, nitrogen, and stable isotope compositions are averaged across both treatments per species (*n*=15).

Site	Species	Tissue	Biomass	Carbon	Nitrogen	$\delta^{15}N$	$\delta^{13}C$
			----g-----	----------%----------		----------‰----------	
F	*Acer rubrum*	Shoot	0.8 (0.1)	44.4 (0.1)	1.18 (0.03)	-0.8 (0.1)	-34.7 (0.2)
		Root	0.5 (0.1)	40.9 (0.8)	1.16 (0.09)	1.0 (0.2)	-34.8 (0.3)
S	*Pinus palustris*	Shoot	3.8 (0.4)	45.7 (0.5)	0.61 (0.02)	-4.6 (0.3)	-31.2 (0.2)
		Root	3.0 (0.3)	42.8 (1.1)	0.29 (0.02)	-3.2 (0.3)	-30.8 (0.2)

Table 5.3. Treatment assignments (*n*) for cores with and without plants at each site, and sampling design used to harvest cores following treatment application.

Treatment	Time T_1 (<1 hour)		Time T_2 (24 hours)	
	(+) Plant	(-) Plant	(+) Plant	(-) Plant
Background	3	2	3	2
Deionized water	3	2	6	2
^{15}N-ammonium sulfate	3	2	6	2
^{15}N-[2]-^{13}C-glycine	0	2	6	2
TOTAL	9	8	21	8

Table 5.4. Coefficients of correlation between soluble organic and inorganic N concentrations ($\mu g\ N\ g^{-1}$) in floodplain soils and sandhills soils (n=20 per site). Significance levels: *$P \leq 0.1$, **$P \leq 0.05$, ***$P \leq 0.001$.

Site		Nitrate-N	Ammonium-N
Floodplain	Soluble organic N	-0.33	0.42**
	Ammonium-N	-0.39**	.
Sandhills	Soluble organic N	0.43**	0.36*
	Ammonium-N	0.77***	.

Table 5.5. Average *in situ* root ^{15}N uptake rates for *A. rubrum* and *P. palustris* plants treated with ^{15}N ammonium sulfate (T_1, *n*=3 per species; T_2, n=6 per species) and ^{15}N-[2]-^{13}C-glycine (T_2, *n*=6 per species). Values in parentheses are one standard error of the mean. †Significant differences were found between T_1 and T_2 ^{15}N ammonium-treated plants only for each species. Uptake rates between species did not differ per treatment.

Site	Species	^{15}N-ammonium†		^{15}N-glycine
		T_1	T_2	T_2
		----------μg ^{15}N g^{-1} d^{-1}----------		
Floodplain	*Acer rubrum* L.	5.6 (1.2)	0.5 (0.1)	0.6 (0.4)
Sandhills	*Pinus palustris* Miller	5.7 (0.5)	1.0 (0.2)	0.5 (0.1)

CHAPTER 6

GENERAL CONCLUSIONS[1]

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[1] Jin, V. L

Most terrestrial ecosystem studies have focused on mineral N as the primary N source for plant growth and as one of the major factors influencing community species composition, structure, and ecosystem productivity and function. Recent research on plant organic N uptake suggests that traditional paradigms of plant N nutrition should be amended to include an organic N perspective. Plant organic N uptake has been documented in almost every ecosystem where it has been investigated and has also been found in a variety of agricultural and managed landscapes. Because of the apparent ubiquity of plant organic N uptake in terrestrial systems, it is critical to explore the roles of organic N in whole-plant, community, and ecosystem processes to further understand and predict how ecosystems function and respond to natural and anthropogenic changes in the environment.

Model results assessing the potential effects of plant organic N uptake on floodplain ecosystem processes indicated that SON uptake by both plant and microbial communities played a key role in nitrogen and carbon cycling times and overall ecosystem productivity. The model predicted that short-term organic N uptake by plants was most affected by competition with soil microorganisms for available N, while longer-term organic N uptake was more strongly affected by soil physicochemical properties. Microbial organic N uptake was largely independent of changes in plant parameters. In addition, sensitivity analyses suggested that C and N dynamics for microbes were relatively less affected by changes in plant parameters while changes in microbial parameters had a more pronounced effect on plant C and N pools.

Overall, seasonal concentrations of SON, ammonium-N, and nitrate-N were highly variable during the two-year study in both forest floor and mineral soils of all ecosystem type and were strongly related to rainfall events. Concentrations of soluble N were greatest in forest floors and decreased with soil depth, and litter dynamics controlling the release of N from forest

floors strongly influenced soluble N concentrations in the mineral soils of all ecosystems. Relationships between both soluble organic and inorganic N and with other soil variables averaged over the entire study revealed the influence of broad environmental gradients on soluble N availability. Our results support published studies showing that patterns in soluble N availability shift with changing spatial and temporal scales, and that SON likely plays a key role in the N dynamics of forest floors and surface mineral soils in these temperate ecosystems.

Finally, model predictions that plant N dynamics were strongly influenced by competition with microbes and that microbial N dynamics were largely independent of plant parameters were confirmed in the field study using stable isotope tracer techniques. The presence of plant roots in soils had no significant effect on gross N mineralization or immobilization rates, nor did the presence of plant roots have an effect on soil concentrations of soluble organic and inorganic N. However, roots of both *Acer rubrum* L. and *Pinus palustris* Miller demonstrated the ability to compete well against soil N immobilization factors for available ammonium-N in floodplain and sandhills soils, respectively, while they did not use N or C applied as the amino acid glycine. Relationships between N concentrations and N mineralization rates were strongly influenced by soil moisture in floodplain soils and differed from patterns of N availability in sandhills soils. In addition, the relationships between soil N availability and plant N uptake were highly variable and differed over time, reflecting the complexity of plant N use patterns. Additions of glycine to both floodplain and sandhills soils influenced plant N uptake and possibly plant productivity by providing easily mineralizable N and C. While organic N uptake as glycine was not unequivocally traced in either tree species examined here, soil SON clearly plays a pivotal role in soil N dynamics and plant N nutrition in these two contrasting temperate ecosystems.